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(71) Applicant: **MERCK & CO. INC.**
126, East Lincoln Avenue P.O. Box 2000
Rahway New Jersey 07065-0900(US)

(72) Inventor: **Donnelly, John J.**
1505 Brierwood Road
Havertown, PA 19083(US)
Inventor: **Liu, Margaret A.**
4 Cushman Rd.
Rosemont, PA 19010(US)
Inventor: **Friedman, Arthur**
121 Froghollow Road
Churchville, PA 18966(US)
Inventor: **Montgomery, Donna L.**
9 Hickory Lane

Chalfont, PA 18914(US)
Inventor: **Hawe, Linda A.**
2610 Skippack Pike
Norristown, PA 19403(US)
Inventor: **Oloff, Allen I.**
1412 Florence Drive
Gwynedd Valley, PA 19437(US)
Inventor: **Shi, Xiao-Ping**
536 Winthrop Rd.
Collegeville, PA 19426(US)
Inventor: **Ulmer, Jeffrey**
128 Dolly Circle
Chalfont, PA 18914(US)
Inventor: **Marshall, Mark S.**
1519 Spruce Ct.
Carmel, Indiana 46032(US)

(74) Representative: **Thompson, John Dr. et al**
Merck & Co., Inc. European Patent
Department Terlings Park Eastwick Road
Harlow, Essex CM20 2QR (GB)

(54) Cellular immunity vaccines from bacterial toxin-antigen conjugates.

(57) Recombinant hybrid proteins having two primary components. The first component is a modified bacterial toxin that has translocating ability, while the second component is a polypeptide or protein that is exogenous to an antigen-presenting cell. The hybrid has the ability to be internalized by an antigen-presenting cell, where the hybrid is subsequently processed and an antigenic segment of the hybrid presented on the surface of the antigen-presenting cell, where the segment elicits an immune response by cytotoxic T lymphocytes.

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Pseudomonas Exotoxin

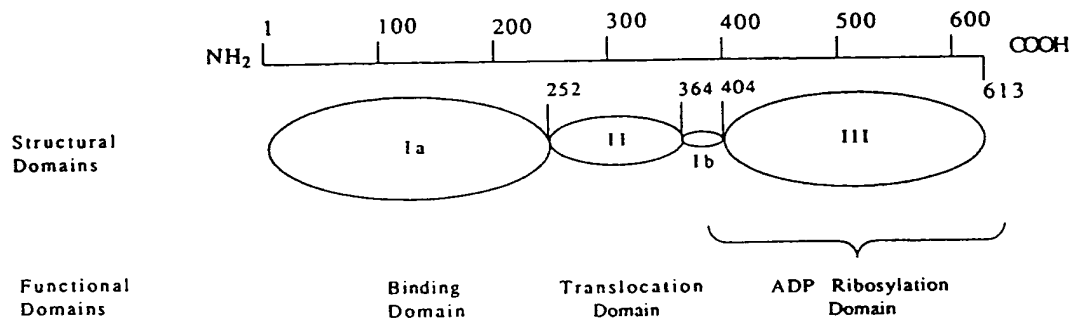


FIG. 1

BACKGROUND OF THE INVENTION

The numerous substances and organisms that threaten the existence of animals having immune systems are either present in extracellular body fluids, such as toxins or bacteria, or else they are harbored within the animal's own cells, such as viruses, certain parasites and oncogene products. This distinction is important to thymus-derived lymphocytes, also known as T cells, which are an important component of vertebrate immune systems. T cells have evolved parallel systems for recognizing intracellular and extracellular antigens. In both systems, antigens are recognized only when they are bound to molecules of the major histocompatibility complex (MHC).

The MHC encodes two types of cell surface molecules that act as receptors for protein antigens. Class I MHC molecules consist of a highly polymorphic integral membrane glycoprotein alpha chain that is noncovalently bound to a beta₂ microglobulin. Class II MHC molecules consist of two noncovalently bound, highly polymorphic, integral membrane glycoproteins. Class I MHC molecules have a groove at the top surface formed by the two amino-terminal domains. The groove holds an antigen. As with other cell surface proteins, during cellular processing in the cytosol, MHC molecules are inserted into the endoplasmic reticulum (ER) and, following chain assembly, are transported to the plasma membrane of the cell via the Golgi complex and post-Golgi complex vesicles.

The recognition of Class I vs. Class II molecules as antigen-presenting sites in general divides T cells into two classes, respectively termed cytotoxic T cells (T_C) and helper T cells (T_H). T_C cells directly lyse cells that are infected with viruses or certain parasites and also will secrete cytokines such as gamma-interferon in order to eradicate intracellular pathogens and tumors.

Virtually all cell types can serve as antigen-presenting cells for T_C cells as long as they express MHC Class I molecules. In general, T_C cells require antigen-presenting cells that are actively biosynthesizing antigen. During processing, the antigen is bound to a nascent Class I molecule in the ER and transported to the plasma membrane via the Golgi complex and post-Golgi complex vesicles. At the plasma membrane, the processed antigen sits in the groove of the MHC Class I molecule, where the processed antigen is available for binding to cell surface receptors of T_C cells. Activation of T_C cells requires interaction between multiple T_C cell surface molecules and their respective ligands on antigen-presenting cells. Once activation has taken place, the lysing and cytokine secretion activity described above can begin.

Antigen processing is the structural modification and trafficking, within the proper subcellular compartments, of protein antigens that enable the determinants recognized by T_C cells to interact with MHC molecules. As noted above, most, and possibly all, somatic cells expressing MHC Class I molecules constitutively process antigens and transport determinants to the cell surface for T_C cell recognition. Antigen processing is thus required for the presentation of intact, folded proteins to T_C cells. Commonly, antigen processing entails the generation of short peptides by cellular proteases, although some intact proteins productively associate with MHC molecules, indicating that proteolysis is not necessarily a component of antigen processing.

Two distinct pathways are used by cells to process antigens. The endosomal pathway is so named because it is accessed through the endosomal compartment. Determinants produced by this pathway usually associate with Class II MHC molecules. The other pathway is the cytosolic pathway. The cytosolic pathway is so named because it can be accessed from the cytosol of the cell by the synthesis of proteins within the cell, or by penetration of plasma or endosomal membranes by extracellular proteins. Such penetration may occur naturally through the fusion of the cell's membrane with a virus, or artificially by osmotic lysis of antigen-containing pinosomes. Determinants produced by cytosolic processing typically associate with Class I MHC molecules. The cytosolic pathway is able to process many different types of foreign proteins for presentation to T_C cells.

Class I MHC molecules associate with antigens in a compartment of the ER. In this regard, it is important to note that the compound Brefeldin A acts by interfering with the normal vesicular traffic between the ER and the Golgi apparatus, and thus also has the effect of blocking the presentation of cytosolically processed antigen on the surface of what would otherwise be an antigen-presenting cell.

It can be seen from the above discussion that, in order to generate response by a cytotoxic T cell, it is generally necessary either to cause the target cell, which has been chosen as an antigen-presenting cell, to endogenously synthesize the protein antigen of interest, or to deliver exogenous protein antigen of interest directly into the cytosolic antigen processing pathway of the target cell. If the latter could be accomplished, a vaccine could be produced which would elicit cytotoxic T cells capable of killing virally or parasitically infected cells or tumor cells, thereby having particular usefulness for preventing three clinical types of diseases.

First, such vaccines could prevent infections caused by viruses such as papilloma or herpes virus which do not undergo a blood-borne phase of infection. This would be especially true in the case of human papilloma virus E7 protein, which is continuously cellularly expressed in the transformed phenotype, and would thus be particularly well suited to attack by sensitized cytotoxic T lymphocytes.

Secondly, there are those infections caused by viruses such as influenza or human immunodeficiency virus (HIV) or parasites whose outer proteins may have high antigenic variability making it difficult to design a vaccine capable of eliciting protective titers of high affinity antibodies with broad specificity. Certain viral internal proteins have less antigenic variation, and peptides derived from such proteins when associated with Class I MHC molecules, would render infected cells susceptible to lysis by sensitized cytotoxic T lymphocytes.

Thirdly, tumors and virally transformed cells express neoantigens that may be presented on Class I MHC molecules, thus rendering these cells suitable targets for cytotoxic T lymphocyte lysis.

Current vaccines generally focus on generating humoral (that is, antibody) responses of the immune system, rather than the cellular immune responses discussed above. Those that do generate cellular immune responses use attenuated live viruses which replicate intracellularly, introducing their constituents into an infected cell's antigen processing pathway as a result of being synthesized within the cell thereby being available for the appropriate protein processing pathway. Thus, there is a need for a non-replicating vaccine that will sensitize cytotoxic T lymphocytes to produce a cellular immune response with a significantly greater margin of safety.

The present invention meets this need by capitalizing on the ability of certain bacterial exotoxins to be internalized into cells through endocytosis via receptors on the cell surface and then translocate out of the resultant endosomes into the cellular compartment in which endogenous proteins are processed for presentation. These exotoxins have been hybridized with polypeptide or protein antigens, which are carried into the cytoplasm and are processed to peptides capable of association with Class I MHC molecules via the physiologic processes discussed above. Once associated with a Class I MHC molecule and presented on the surface of the antigen-presenting cell, they can sensitize cytotoxic T lymphocytes against other infected cells synthesizing the same polypeptide or protein. By virtue of these actions, the invention presents vaccines which can be effective in prophylaxis against viruses, parasites and malignancies.

It is an additional object of the present invention to produce hybrid proteins of certain bacterial exotoxins having translocation domains, hybridized with polypeptides or proteins selected for their antigenic activity, which hybrids will be useful as probes for studying the intracellular processing and subsequent presentation of endogenously synthesized cytoplasmic proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structural domains of Pseudomonas exotoxin, along with the numbers of the amino acid residues that define the known limits of the structural domains. Amino acid residues are numbered as defined in Gray, et al, PNAS USA 81 = 2645-2649(1984).

Figure 2 is a restriction map for plasmid pVC45-DF + T.

Figure 3 is a restriction map for plasmid pBluescript II SK.

Figure 4 is a restriction map for plasmid pBR322.

Figure 5 is a graph showing the results of using hybrid construct PEMA in immunologically sensitizing U-2 OS cells, a human cell line.

Figure 6 shows that a hybrid protein made of the binding and translocating domains of Pseudomonas exotoxin and a peptide epitope of influenza A matrix protein can competitively prevent the intact Pseudomonas exotoxin from binding to and killing target cells.

SUMMARY OF THE INVENTION

The invention is a hybrid protein of two species, the first species being a modified bacterial toxin that has a translocating domain. The second species is a polypeptide or protein. The polypeptide or protein is exogenous to an antigen-presenting cell of interest. The hybrid of the bacterial toxin and the exogenous polypeptide or protein are constructed in such a way as to be capable of eliciting an immune response by cytotoxic T lymphocytes.

A preferred bacterial toxin is a modified Pseudomonas exotoxin. Pseudomonas exotoxin is known to consist of four structural domains, namely Ia, II, Ib and III. This is shown at Figure 1, along with the numbers of the amino acid residues that define the known limits of the structural domains. More preferably, the Pseudomonas exotoxin is modified by deletion of structural domain III, that is the ADP-ribosylating structural

domain, although alternatively domain III need not be entirely deleted, but may rather be sufficiently altered in its amino acid sequence so as to render it enzymatically nonfunctional as an ADP-ribosylating enzyme. Most preferably, the modified bacterial toxin has only a cellular recognition domain and a translocating domain, (with or without the 5 C-terminal amino acids of Domain III added to the C-terminus of the polypeptide or protein antigen), or even just the translocating domain with or without targeting ligand. In the case of *Pseudomonas* exotoxin, the cellular recognition domain and translocating domain are known to exist within structural domains Ia, II and Ib. Also most preferably, modified *Pseudomonas* exotoxins are arranged on the amino-terminal side of the hybrid, while the exogenous polypeptide or protein is arranged on the carboxyl-terminal side of the hybrid.

The exogenous polypeptide or protein, which is exogenous to an antigen-presenting cell of interest, is preferably a polypeptide or protein of viral origin. More preferably, the viral polypeptide is a viral protein fragment, and most preferably is taken from the group comprising the matrix protein of influenza A virus; residues 57 to 68 of the matrix protein of influenza A virus (the matrix epitope known to bind MHC HLA-A2); the nucleoprotein of influenza A virus; or the GAG protein of human immunodeficiency virus-1.

Functionally, the hybrid is capable of eliciting an immune response by cytotoxic T lymphocytes, by virtue of being at least partially presented on an antigen-presenting cell surface. More specifically, the hybrid functionally is capable of being internalized by an antigen-presenting cell and further capable of being processed, via the endogenous protein processing pathway, on its way to at least partial presentation on the surface of the antigen-presenting cell.

The hybrid proteins preferably will use polypeptide or protein antigens for use as a vaccine, and most preferably will use viral antigens. Most preferably, these viral antigens will be conserved viral proteins. The hybrids will be incorporated in an amount sufficient to elicit an immune response by cytotoxic T lymphocytes into vaccines further comprising pharmaceutically acceptable carriers. The vaccines will be sufficient to immunize a host against the diseases influenza, acquired immunodeficiency syndrome, human papilloma virus, cytomegalovirus, Epstein-Barr virus, Rota virus, and respiratory syncytial virus, tumors and parasites.

The present invention further relates to recombinant DNA segments containing nucleotide sequences coding for the fused proteins described above, as well as plasmids and transformants harboring such recombinant DNA segments, as well as methods of producing the hybrid proteins using such recombinant DNA segments and methods of administration of the hybrid proteins as vaccines to hosts.

DETAILED DESCRIPTION OF THE INVENTION

The term "translocating domain" shall mean a sequence of amino acid residues sufficient to confer on a polypeptide or protein the ability to translocate across a cell membrane into a cellular compartment for processing endogenous proteins.

The term "exogenous to an antigen-presenting cell" shall mean polypeptides that are not encoded by the unmutated genome of a given antigen-presenting cell.

The term "antigen-presenting cell" shall refer to a variety of cell types which carry antigen in a form that can stimulate cytotoxic T lymphocytes to an immunologic response.

The term "immune response" shall mean those cytotoxic processes of cell lysis and cytokine release engaged in by cytotoxic T lymphocytes that have been stimulated by antigen presented by an antigen-presenting cell. This term shall also include the ability of a host's cytotoxic T lymphocytes to retain their cytotoxic response to subsequent exposure to the same antigen that will lead to more rapid elimination of the antigen than in a non-immune state.

The term "presented on an antigen-presenting cell surface" shall mean that process by which an antigen is seated within a ligand site of a major histocompatibility complex Class I protein on the surface of an antigen-presenting cell.

The term "being internalized by an antigen-presenting cell" shall mean the process of endocytosis resulting in endosome formation.

The term "cellular recognition domain" shall mean a sequence of amino acid residues in a polypeptide sufficient to confer on that polypeptide the ability to recognize a receptor site on the surface of a target cell.

The term "ADP ribosylating domain" shall mean a sequence of amino acids sufficient to confer on a polypeptide the ability to modify elongation factor II within a cell, and thereby severely impair the viability of the cell or kill it.

The term "vaccine" shall mean a pharmaceutically acceptable suspension of a given therapeutic entity administered for the prevention, amelioration or treatment of infectious diseases.

The term "conserved viral protein" shall mean those viral proteins that do not vary from strain to strain of a given species of virus, or to those viral proteins that are generally unlikely to undergo mutation as a function of time in a given strain.

The term "arranged on the amino terminal side of said hybrid" shall mean that a peptide sequence has been inserted at any point between the amino terminus of a hybrid and the hybrid's middle amino acid residue.

The term "arranged on the carboxy terminal side of said hybrid" shall mean that a peptide sequence has been inserted at any point between the carboxy terminus of a hybrid and the hybrid's middle amino acid residue.

The hybrid proteins of the present invention are fusion protein constructs of a bacterial toxin having a translocating domain fused to a polypeptide or protein that has been selected for its antigenicity for a given disease, as well as for being exogenous to a targeted antigen-presenting cell. A preferred bacterial toxin is the *Pseudomonas* exotoxin. This exotoxin is known to comprise four structural domains, as shown in Figure 1. These domains are designated Ia, II, Ib and III. Structural domain Ia is known to be necessary for binding of the exotoxin to a receptor site on the surface of a target cell. Structural domain II is known to be necessary for translocation of the exotoxin across an internal membrane the targeted cell. Part of structural III are known to be an ADP ribosylating enzyme that bind to the protein Elongation Factor 2, which generally results in the death of the target cell.

In a preferred embodiment of the present invention, structural domain III (or all domain III except for the C-terminal amino acids) has been deleted from the *Pseudomonas* exotoxin molecule, and has been replaced with one of several polypeptides or proteins chosen for their ability to act as antigens and therefore be useful as vaccines. The antigens used for vaccines include antigens of viruses whose hosts are higher vertebrates, such as antigen of influenza A virus, human immunodeficiency virus-1, human papilloma virus, cytomegalovirus, Epstein-Barr virus, Rota virus, and respiratory syncytial virus. Other viruses include herpes viruses such as herpes simplex virus, varicella-zoster virus, adult T cell leukemia virus, hepatitis B virus, hepatitis A virus, parvoviruses, papovaviruses, adenoviruses, pox viruses, reoviruses, paramyxoviruses, rhabdoviruses, arena-viruses, and coronaviruses. Other disease states can have antigens designed for them and used in alternative embodiments of the present invention, including antigens with pathogenic protozoa, such as malaria antigen.

The fusion proteins of the present invention are preferably manufactured through expression of recombinant DNA sequences.

The DNAs used in the practice of the invention may be natural or synthetic. The recombinant DNA segments containing the nucleotide sequences coding for the embodiments of the present invention can be prepared by the following general processes:

- (a) A desired truncated gene is cut out from a plasmid in which it has been cloned, or the gene can be chemically synthesized;
- (b) An appropriate linker is added thereto as needed, followed by construction of a fused gene; and
- (c) The resulting fused protein gene is ligated down stream from a suitable promoter in an expression vector.

Techniques for cleaving and ligating DNA as used in the invention are generally well known to those of ordinary skill in the art and are described in Molecular Cloning, A Laboratory Manual, (1989) Sambrook, J., et al., Cold Spring Harbor Laboratory Press.

As the promoter used in the present invention, any promoter is usable as long as the promoter is suitable for expression in the host used for the gene expression. The promoters can be prepared enzymatically from the corresponding genes, or can be chemically synthesized.

Conditions for usage of all restriction enzymes were in accordance with those of the manufacturer, including instructions as to buffers and temperatures. The enzymes were obtained from New England Biolabs, Bethesda Research Laboratories (BRL), Boehringer Mannheim and Promega.

Ligations of vector and insert DNA's were performed with T4 DNA ligase in 66mM Tris-HCl, 5mM MgCl₂, 1mMDTE, 1mMATP, pH 7.5 at 15°C for up to 24 hours. In general, 1 to 200 ng of vector and 3-5x excess of insert DNA were preferred.

Selection of *E. coli* containing recombinant plasmids involve streaking the bacteria onto appropriate antibiotic containing LB agar plates or culturing in shaker flasks in LB liquid (Tryptone 10g/L, yeast extract 5g/L, NaCl 10g/L, pH 7.4) containing the appropriate antibiotic for selection when required. Choice of antibiotic for selection is determined by the resistance markers present on a given plasmid or vector. Preferably, vectors are selected by ampicillin.

Culturing of *E. coli* involves growing in Erlenmeyer flasks in LB supplemented with the appropriate antibiotic for selection in an incubation shaker at 250-300 rpm and 37°C. Other temperature from 25°C-

37°C could be utilized. When cells are grown for protein production, they are induced at $A_{560} = 1$ with IPTG to a final concentration of 0.4 mM. Other cell densities in log phase growth can alternatively be chosen for induction.

Harvesting involves recovery of *E. coli* cells by centrifugation. For protein production, cells are harvested 3 hours after induction though, other times of harvesting could be chosen.

In the present invention, any vector, such as a plasmid, may be used as long as it can be replicated in a procaryotic or eucaryotic cell as a host.

By using the vector containing the recombinant DNA thus constructed, the host cell is transformed via the introduction of the vector DNA.

The host cell of choice is BL21 (DE3) cells (*E. coli*), obtained from F. Wm. Studier, Brookhaven National Laboratories, Stony Brook, N.Y. Reference is also made to Wood, J. Mol. Biol., 16:118-133 (1966) U.S. Patent No. 4,952,496, and Studier, et al., J. Mol. Biol. 189:113-130 (1986). However, any strain of *E. coli* containing an IPTG inducible T7 polymerase gene would be suitable. For routine cloning, *E. coli* strain DH5 α (BRL) can be used.

BL21(DE3) strain of *E. coli* was acquired under license from W. F. Studier. Reference is made to Studier, W. F. et. al., Methods in Enzymology, Vol. 185, Ch. 6, pp 60-89 (1990). This strain is unique to the extent that it contains an inducible T7 polymerase gene. The strain has no amino acid, sugar or vitamin markers, so it can grow on any rich or defined bacterial medium. It can be grown between 25°C and 37°C. It needs aeration, and it needs IPTG for induction of the T7 polymerase.

In the present invention, the fused proteins can be separated and purified by appropriate combinations of well-known separating and purifying methods. These methods include methods utilizing a solubility differential such as salt precipitation and solvent precipitation, methods mainly utilizing a difference in molecular weight such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electric charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatography, methods utilizing a difference in hydrophobicity such as reverse-phase high pressure liquid chromatography, methods utilizing a difference in isoelectric point, such as isoelectrofusing electrophoresis, and methods using denaturation and reduction and re-naturation and oxidation.

Preferred embodiments of the invention will now be described in detail in the following non-limiting examples. The most preferred embodiments of the invention are any or all of those specifically set forth in these examples. These examples are not, however, to be construed as forming the only genus that is considered as the invention, and any combination or sub-combination of the examples may themselves form a genus. These examples further illustrate details for the preparation of various embodiments of the present invention. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these embodiments.

EXAMPLE 1

BS-PEM1-2

A 1.3kb *Nru*I/*Sac*II fragment of plasmid pVC45-DF+T (Fig. 2) (obtained from Dr. Ira Pastan of the National Institute of Health) containing the domain I and II coding regions of *Pseudomonas* exotoxin (PE) (Sequence ID No. 1) was subcloned into pBluescript II SK (Stratagene, Fig. 3) restricted with *Hinc*II and *Sac*II. The resulting construct is designated BS-PE. The influenza M1 (M1) gene (Sequence ID No. 2 and 3) which codes for the matrix protein of influenza A virus was subcloned into BS-PE restricted with *Sac*II and *Sac*I by amplifying the M1 gene from pApr701 (P. Palase, Mt. Sinai Medical Center, New York, N.Y. pApr 701 consists of the M1 gene cloned into the *ECOR*I site of pBR322, shown at Fig. 4. Reference is made to Young, J.F. et. al, Expression of Influenza Virus Genes; The Origin of Pandemic Influenza Virus; 1983) by polymerase chain reaction (PCR) (Gene Amp® PCR Reagent Kit; Perkin Elmer Cetus, Norwalk, Conn. 06859) with oligonucleotide primers which added a *Sac*II site adjacent to M1 codon number 2 (Sequence ID No. 4) and a *Sac*I site 3' of the M1 termination codon (Sequence ID No. 5). This plasmid is designated BS-PEM1-1.

The truncated *ompA* leader coding sequence was removed from the 5' end of the fusion gene by replacing the small *Xho*I/*Hind*III fragment of BS-PEM1-1 with the oligonucleotide sequence shown in Sequence ID No. 6. The resulting plasmid is named BS-PEM1-2 and encodes a fusion gene consisting of *Pseudomonas* exotoxin amino acids 2 through 414 joined to M1 amino acids 2 to 252 (Sequence ID No. 7 and 8).

EXAMPLE 2

pVC-ompA-PEM1-2

5 pVC45DF + T vector was prepared by restriction digestion with HindIII and EcoRI, followed by gel purification.

The PEM1 insert fragment was prepared by restriction digestion of BS-PEM1-1 with SacI, followed by T4 DNA polymerase treatment to remove the 3' overhang. EcoRI linkers were added to the blunted SacI site, followed by restriction digestion with HindIII. The HindIII-EcoRI fragment was gel purified (Molecular Cloning Manual, Gene Clean Kit, Bio 101, Inc. P.O. Box 2284, La Jolla, CA 92038) and ligated into the
10 prepared pVC45-DF + T vector. The resulting construct was named pVC-ompA-PEM1-2.

The ompA signal sequence was removed from the construct by restriction digestion of pVC-ompA-PEM1-2 with XbaI and HindIII. An oligonucleotide fragment containing the T7 promoter, ribosome binding site and initiation sequence was ligated into the vector whose base sequence is shown at Sequence ID No.
15 9. The resulting plasmid construct was named pVC-PEM1-2 and encodes a T7 polymerase-driven gene fusion consisting of PE amino acids 2 through 414 joined to influenza M1 amino acids 2 through 252. The 5' and 3' ends of the coding region, as well as the PE to M1 fusion site and cytotoxic T lymphocyte epitope coding sequences (Rotzschke, O. et. al., Nature 348, 252 (1990) were confirmed by DNA sequencing.

EXAMPLE 3

BS-PEMa

The influenza Ma sequence (coding for residues 57-68 of the influenza matrix protein) was obtained by
25 amplifying a portion of the influenza M1 gene in pApr701 by polymerase chain reaction (PCR) with oligonucleotide primers which added a SacII site adjacent to influenza M1 codon No. 57 (Sequence ID No. 10) and a termination codon and a SacI site 3' of the M1 codon No. 68 (Sequence ID No. 11). This fragment was cut with SacII and SacI and subcloned into BS-PE digested with SacII and SacI. The resulting plasmid is named BS-PEMa-1 and was verified by sequencing through the junctions and the Ma sequence itself.

EXAMPLE 4

Subcloning of PEMa from BS-PEMa1 into PVC45DF + T

35 The PEMa insert (Sequence ID No. 12) was prepared by restricting BS-PEMa-1 with SacI and removing the 3' overhang by treatment with T4 DNA polymerase, then restricting with ApaI and gel purifying.

pVC45DF + T was restricted with EcoRI and the 5' overhang filled in with Klenow enzyme treatment (Molecular Cloning Manual, ibid.). It was subsequently restricted with ApaI and gel purified. The vector and fragment were ligated together, and the resulting construction was named pVC-ompA-PEMa-1. The
40 construction was verified by sequencing across the junctions and through Ma.

The ompA leader sequence was removed from pVC-ompA-PEMa-1 by digestion with XbaI and HindIII. An oligonucleotide fragment containing the T7 promoter, ribosome binding site, initiation sequence and a build-back of the 5' end of the PE coding region (Sequence ID No. 13) was ligated to the vector. The resulting construction was named pVC-PEMa-1 and encodes a T7 polymerase driven gene fusion consisting
45 of PE amino acids 2 to 414 joined to influenza M1 amino acids 57 to 68 (Ma) Sequence ID No. 14 and 15. The 5' end of pVC-PEMa-1 was verified by sequencing through the oligonucleotide fragment.

EXAMPLE 5

50 Construction of pVC-PEBT

A control plasmid was constructed which encodes a T7 polymerase driven gene fusion consisting of PE amino acids 2 to 414 followed by termination codons. pVC-PEM1-2 was digested with SacII and EcoRI to remove the M1 sequence. The vector was gel purified and ligated to an oligonucleotide that builds back PE
55 codon No. 414 followed by termination signals shown in Sequence ID No. 16. The resulting construction was named pVC-PEBT (Sequence ID No. 17 and 18) and was verified by sequencing across the junctions and the oligonucleotide addition.

EXAMPLE 6

BSK-PEM1

5 BSK-PEM1 was made from BS-PEM1 by the replacement of the 21 base pair XhoI/HindIII fragment with a 24 base pair fragment encoding a consensus eucaryotic ribosome binding site (Sequence ID No. 19). The purpose of the construct was to increase the yields of in vitro translated PEM1 protein. Thus, an additional object of the invention is to increase yields of translated PEM1 protein.

10 EXAMPLE 7

pVCPE/2 (pVC45DF + T/2)

pVCPE/2 was made by replacing the 105 base pair PpuMI/EcoRI fragment of pVC45DF + T with a 46
15 base pair DNA fragment encoding an inframe duplication of PE codons 604 to 613 flanked by unique cloning sites (Sequence ID No. 20). This construct is used for generating full-length molecules of PE with the deletion of residue 553 resulting in an inactivated toxin domain (Sequence ID No. 21 and 22) fused to protein segments of choice between PE codons 604 and 605. One may replace the ompA signal sequence with the promoter/ribosome binding site as described for PVC-PEM1-2.

20 EXAMPLE 8

pVCPE/2-Ma

25 pVCPE/2-Ma was made by ligating into the XmaI site of pVCPE/2 a 48 base pair DNA fragment encoding amino acids 55 through 67 (Sequence ID No. 23). This construct expresses in E. coli full-length PE with M1 amino acids 55 through 67 inserted between PE amino acid 604 and 605 (Sequence ID No. 24 and 25). One may replace the ompA signal sequence with the promoter/ribosome binding site as described for pVC-PEM1-2.

30 EXAMPLE 9

pVCPE/2-M1:15-106

35 pVCPE/2-M1:15-106 was made by subcloning a PCR-amplified DNA fragment encoding M1 amino acids 15 through 106 into the XmaI site of pVCPE/2. The sequence of the oligonucleotide primers used to amplify the M1 segment are those shown at Sequence ID No. 26 and 27, respectively. This construct expresses in E. coli full length PE with M1 amino acids 15 through 106 inserted between PE amino acid 604 and 605 (Sequence ID No. 28 and 29). One may replace the ompA signal sequence with the
40 promoter/ribosome binding site as described for pVC-PEM1-2.

EXAMPLE 10

pVCPEde1(403-613)

45 pVCPEde1(403-613) was made by restricting pVC45DF + T with SacII followed by elimination of the 3' SacII overhang with T4 DNA polymerase and the ligation of a 3-frame termination linker whose nucleic acid sequence is given at Sequence ID No. 30. This construct will express FE domains I, II and Ib only, fused to the ompA leader in E. coli.

50 EXAMPLE 11

pVCPEde1(403-505)

55 pVCPEde1(403-505) was made by restricting pVC45DF + T with SacII and XhoI followed by removal of restriction overhangs with mung bean nuclease (New England Biolabs). The vector fragment was recovered and reclosed with DNA ligase. This construct will express in E. coli the PE protein lacking amino acids 403 through 505.

EXAMPLE 12

pVCPEde1(494-505)

- 5 pVCPEde1(494-505) was made by restricting pVC45DF + T with BamHI and XhoI followed by the filling in of the 5' overhangs with Klenow fragment. The vector fragment was recovered and reclosed with DNA ligase. This construct will express in E. coli the PE protein lacking amino acids 494 through 505.

EXAMPLE 13

10 pVCPEde1(494-610)

- pVCPEde1(494-610) was made by restricting PVC45DF + T with BamHI and PpuMI followed by the filling in of the 5' overhangs with Klenow fragment. The vector fragment was recovered and reclosed with
 15 DNA ligase. This construct will express in E. coli the PE protein lacking amino acids 494 through 610. All of the pVCPEde1 plasmids were useful in determining to what extent the toxin domain of PE could be truncated without resulting in the expression of an insoluble protein in E. coli. It thus became an additional object of the invention to provide hybrids having the minimal toxin domain of PE that would retain water solubility.

EXAMPLE 14

Addition of Sequences Between pE and M1 in pVC-PEM1-2

- 25 Oligonucleotide linkers can be added at the SacII site between PE and M1 in pVC-PEM1-2. These linkers can be designed to add cleavage sites and/or signal sequences which can help the M1 portion of the fusion protein to become available for presentation within the cell. SacII digestion cleaves the gene between the last two PE codons (for amino acids 413 and 414) and provides an appropriate site for such additions.
 The following four constructions have been made by inserting linkers at the SacII site. The constructions
 30 have been verified by sequencing across the SacII junctions and through the complete linker.

EXAMPLE 15

pVC-PE-RK-M1

- 35 This vector contains an ARG LYS(RK) cleavage site inserted into the SacII site, using an oligonucleotide linker as shown in Sequence ID No. 31. The resulting amino acid sequence between amino acids 413 and 414 of PE is Gly Gly Arg Lys Ser.

EXAMPLE 16

pVC-PE-RKSigl-M1

- 45 This vector contains an ARG LYS(RK) cleavage site and the signal sequence that is shown in Sequence ID No. 32 from the Influenza A hemagglutinin (HA) protein inserted at the SacII site, using the oligonucleotide linker disclosed at Sequence ID No. 33. The resulting amino acid sequence between amino acids 413 and 414 of PE is also as shown in Sequence ID No. 34.

EXAMPLE 17

PVC-PE-Sig1-M1

- 50 This vector contains the signal sequence of HA without the RK cleavage site inserted into the SacII site using the oligonucleotide linker shown at Sequence ID No. 35. The resulting amino acid sequence between
 55 amino acids 413 and 414 of PE is also as shown at Sequence ID No. 36.

EXAMPLE 18

pVC-PE-Sig2-M1

5 This vector contains the signal sequence shown at Sequence ID No. 37, derived from amino acids 22 to 48 from ovalbumin inserted into the SacII site, using the oligonucleotide linker of Sequence ID No. 38. The resulting amino acid sequence between amino acids 413 and 414 of PE is also as that shown in Sequence ID No. 39.

10 Addition of Sequences Between PE and Ma In pVC-PEMa-1

Oligonucleotide linkers can be added at the SacII site between PE and Ma in pVC-PEMa-1. These linkers can be designed to add cleavage sites and/or signal sequences which can help the Ma peptide to become available for presentation within the cell. SacII digestion cleaves the gene between the last two PE
15 codons (for amino acids 413 and 414) and thus provides an appropriate site for such additions.

The following four examples have been made by inserting linkers at the SacII site. The constructions have been verified by sequencing across the SacII junctions and through the complete linker.

EXAMPLE 19

20 pVC-PE-RKSig1-Ma

This vector contains an ARG LYS (RK) cleavage site and the signal sequence from the Influenza A hemagglutinin (HA) protein inserted into a blunted SacII site, using the oligonucleotide linker shown at
25 Sequence ID No. 40. The resulting amino acid sequence between amino acids 413 and 414 of PE exotoxin is also as shown at Sequence ID No. 41.

EXAMPLE 20

30 pVC-PE-Sig1-Ma

This vector contains the single sequence of HA without a cleavage site inserted into a blunted SacII site using the oligonucleotide linkers shown in Sequence ID No. 42. The resulting amino acid sequence between amino acids 413 and 414 of PE is also as shown in Sequence ID No. 43.

35 EXAMPLE 21

pVC-PE-Sig2-Ma

40 This vector contains a signal sequence derived from amino acids 22 through 48 from ovalbumin inserted into a blunted SacII site, using the oligonucleotide linker as seen in Sequence ID No. 44. The resulting amino acid sequence between amino acids 413 and 414 of PE is also as shown in Sequence ID No. 45.

45 EXAMPLE 22

pVC-PE-Sig1Sig2-MA

This vector contains the signal sequence derived from HA, followed by the signal sequence from
50 ovalbumin inserted into the SacII site, using the oligonucleotide linker shown at Sequence ID No. 46. The resulting amino acid sequence between amino acids 413 and 414 of PE is also as shown at Sequence ID No. 47.

55

EXAMPLE 23

BSPEM1c5aa

5 The plasmid BSPEM1-2 was digested with SacI and StuI and ligated to the oligonucleotide linker shown at Sequence No. 48. This linker builds back the C-terminus of the M1 protein and adds the last five amino acids from the C-terminus of the PE protein, whose sequence is Arg Glu Asp Leu Lys, followed by a termination codon. This also incorporates an EcoRI site. The resulting plasmid was named BSPEM1c5aa and was sequenced across the junctions (Sequence ID No. 49 and 50) and the linker for verification of the
10 construction.

EXAMPLE 24

pVC-PEM1c5aa

15 The plasmid BSPEM1c5aa was digested with HindIII and EcoRI and 1.8 kb PEM1c5aa fragment was gel purified. The plasmid pVC-PEM1-2 was digested with HindIII and EcoRI and the 3.2 kb vector fragment was ligated to the 1.8 kb PEM1c5aa fragment and the resulting plasmid was named pVC-PEM1c5aa. The 5' and 3' ends of the PEM1c5aa insert were verified by sequencing.

EXAMPLE 25

pVC-PENPc5aa

25 A fragment containing the nucleoprotein (NP) of Influenza A virus was obtained from plasmid pApr501 (obtained from Peter Palase, Mt. Sinai Medical Center, New York, N.Y. pApr501 is said nucleoprotein gene cloned into the EcoRI site of pBR322, (Fig. 4) by polymerase chain reaction with oligonucleotide primers which added a SacII site adjacent to the ATG codon of NP to give the sequence shown at Sequence ID No. 51, and the last 5 amino acids of FE followed by a termination codon and an EcoRI site to the 3' end of NP
30 to give the sequence shown at Sequence ID No. 52. The polymerase chain reaction fragment was digested with SacII and EcoRI and ligated to the plasmid pVC-PEM1-2 digested with SacII and EcoRI. The resulting plasmid is named pVC-PENPc5aa. The 5' and 3' ends of the PENPc5aa insert (Sequence ID No. 53 and 54) were verified by sequencing. This construction fuses the binding and translocation domains of PE to the Influenza A nucleoprotein.

EXAMPLE 26

pVC-ompA-PEGAG

40 The HIV GAG gene was obtained from plasmid HIVpBR322 (obtained from Ron Diehl Merck, Sharpe and Dohme Research Laboratories, West Point, PA., Fig. 5) by polymerase chain reaction with oligonucleotides that added a SacII site adjacent to the ATG codon of GAG to give the nucleotide sequence shown at Sequence ID No. 55, and a SacI site immediately after the termination codon at the 3' end to give the nucleotide sequence at Sequence ID No. 56. The polymerase chain reaction fragment was digested with
45 SacII and ligated to plasmid pVC45DF + T, which had been digested with EcoRI, the 5' overhang filled in by Klenow fragment, and digested with SacII. The resulting plasmid was named pVC-ompA-PEGAG (Sequence ID No. 57 and 58) and was verified by a partial sequence at the SacII junction. This construction fused the binding and translocation domains of FE to the GAG gene of HIV-1 virus. The fusion protein contains an ompA leader sequence. Alternatively, any vector containing the complete coding
50 region for HIV GAG can be used with these oligomers to generate the HIV GAG gene by PCR.

EXAMPLE 27

Expression of PEM1, PEMa and PEBT

55 Frozen competent BL21(DE3) cells (as described by Studier, et al. Mol. Biol., 189, 113-130, 1986) were prepared as described (DNA cloning, Vol. 1, p. 121, Ed. D N Glover, IRL Press, Wash., D.C.).

BL21(DE3) cells were transformed with pVC-PEM1-2, pVC-PEMa-1, or pVC-PEBT as described below (this can be performed with pVC-PE fusion plasmids in general) and transformants were selected on L-Amp plates. Fresh transformants were used to inoculate L-Amp liquid cultures at A560 = 0.1. Cultures were grown at 37 °C with vigorous aeration and induced at A560 = 1.0 with IPTG to a final concentration of 0.4 mM. Cultures were harvested after 3 hours of induction and the cell pellets used for protein extraction and purification (Protein Structure: A Practical Approach, T.E. Creighton, ed., IRL Press at Oxford Univ. Press, Ch. 9, 191 (1989)).

Transformation Procedure

A bath of dry ice/ethanol was prepared and maintained at -70 °C. Competent cells were removed from a -70 °C freezer and thawed on ice. A sufficient number of 17 x 100 mm polypropylene tubes (Falcon 2059) were placed on ice. 100 µl aliquots of gently mixed cells were prepared in the chilled polypropylene tubes. DNA was added by moving a pipette through the cells while dispensing; the cells were then gently shaken for 5 seconds after addition. The cells were incubated on ice for 30 minutes, then heat-shocked in a 42 °C water bath for 45 seconds without shaking. The cells were again placed on ice for 2 minutes. 0.9 ml of S.O.C. reagent (Bactotryptone 2%, Yeast Extract 0.5%, NaCl 10mM, KCl 2.5mM, MgCl₂ · MgSO₄ 20mM, Glucose 20mM and distilled water, up to 100 ml) was added and the mixture shaken for 1 hour at 225 rpm and 37 °C, then plated on antibiotic plates, spread gently.

EXAMPLE 28

Incubation of U-2 OS Cells With ⁵¹Cr and Protein/PEMa

U-2 OS cells (ATCC) were harvested from flasks, after a 1X wash with RCM 8, using 1mM EDTA. The flasks were incubated at 37 °C for 10 minutes until cells were nonadherent. Five ml. of U-2 OS medium [McCoy's 5A (GIBCO) supplemented with 15% fetal bovine serum (HyClone) and penicillin 100 U/ml and streptomycin 100 µg/ml (GIBCO)] was added, and the cells were centrifuged for 10 minutes at 210 x g.

Cells were resuspended in U-2 OS medium at 8.5 x 10⁵/ml. To each well of a 12-well plate, 0.7 ml of cell suspension was added. Negative controls include U-2 OS medium alone and PEBT. The positive control for sensitization of U-2 OS cells is KKAM1 (2 µg/ml), from M. Gammon and H. Zweerink (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ). PEMa was added at 0.2µM or greater well concentration. Simultaneously, 137.5 µCi of ⁵¹Cr (Amersham) was added to each well. Medium was added to all wells to bring the total volume to 1 ml. This was placed at 37 °C, 5.5% CO₂ for 14 hours.

EXAMPLE 29

Assay Protocol for CTL Activity Against Sensitized U-2 OS Targets

After the 14 hour incubation, U-2 OS were removed, after a 1X RCM 8 wash using 1mM EDTA. Plates were incubated at 37 °C for 10 minutes until cells were nonadherent. K medium [RPM1 1640 (GIBCO) supplemented with 10% fetal bovine serum (HyClone), 10 mM HEPES (GIBCO), 2 mM L-glutamine (GIBCO), penicillin 100 U/ml and streptomycin 100 µg/ml (GIBCO), and 50 µM 2-mercaptoethanol (Bio-Rad)] was added to give a total volume of 10 ml; cells were centrifuged for 10 minutes at 210 x g. The cells were incubated at room temperature for 10 minutes in 10 ml of K medium before entering the second centrifugation. The cells were then resuspended in 1 ml of K medium, counted, and resuspended to 1 x 10⁵/ml in K medium.

Human cytotoxic T lymphocytes, generated from one donor, were harvested, centrifuged for 10 minutes at 92 x g, and resuspended in K medium at 2.5 x 10⁶/ml.

100 µl of human CTLs were added to each well of a 96-well U-bottom microtiter plate (CoStar). 100 µl of the U-2 OS ⁵¹Cr-labeled targets were also added to these wells for a final effector/target ratio of 25:1. Spontaneous ⁵¹Cr release was determined by incubating U-2 OS cells with 100 µl of K medium alone. The maximal release was determined by adding 100 µl of 6 M HCl to 100 µl of targets. The plates were quickly centrifuged to bring down the cells, and incubated for 2 hours at 37 °C.

After this 2 hour incubation, the plates were centrifuged for 5 minutes, 330 x g, 5 °C; 30 µl of supernatant was harvested from each well onto a plastic-backed filtermat (Pharmacia/LKB). The mat was dried in the microwave for 3 minutes on medium-high power. The mat was placed into a sample bag with 10 ml of BetaPlate Scint, heat sealed and placed into the BetaPlate 1205 counter (Pharmacia/LKB). Results

were expressed as % specific lysis, defined as:

$$\% \text{ specific lysis} = \frac{\text{Experimental} - \text{Spontaneous}}{\text{Maximal} - \text{Spontaneous}} \times 100$$

where

Experimental = counts per minute from the 30 μ l of supernatant harvested from the wells containing targets plus human cytotoxic T lymphocytes, as determined by a Betaplate 1205 counter;

10 Spontaneous = counts per minute from the 30 μ l of supernatant harvested from the wells containing targets plus medium alone, as determined by the BetaPlate 1205 counter; and

Maximal = counts per minute from the 30 μ l of supernatant harvested from the wells containing target plus 6M HCl (Fisher Scientific), as determined by the BetaPlate 1205 counter.

Results are presented graphically in Fig. 5, with U-2 OS medium alone and PEBT as negative controls, 15 and KKAM1 as a positive control. Greater than 10% specific lysis is considered a positive response (Cerottini, et.al., J. Exp. Med. 140:703, 1974).

EXAMPLE 30

20 Generation of M1-specific Human Cytotoxic T Lymphocytes

Original stock of human cytotoxic T lymphocytes was derived by harvesting blood from one donor into a syringe (Becton Dickinson) containing 25 U of heparin for each ml of whole blood (Elkins-Sinn, Inc.). The heparinized blood was pipetted directly into a Leucoprep tube (Becton Dickinson) and centrifuged for 20 25 minutes at 1700 X g. The buffy coat which was seen just above the interface was removed, centrifuged for 10 minutes at 92 X g, and washed twice in RPMI 1640 (GIBCO). The peripheral blood mononuclear cells (PBLs) recovered from the Leucoprep procedure were resuspended in 10 ml of CTL medium [RPMI 1640 (GIBCO) supplemented with 10% donor or pooled human plasma, 4 mM L-glutamine, 10 mM HEPES, penicillin 100 U/ml and streptomycin 100 μ g/ml (GIBCO)] at 1×10^6 /ml.

30 M1 peptide (received from M. Gammon and H. Zweerink, MSDRL, Rahway; 2 mg/ml stock) in DMSO was diluted 1:10 in RPMI 1640 (GIBCO). M1 peptide was added to the 10 ml of lymphocytes at a final concentration of 5 μ g/ml. The cells were then plated at 1.5×10^6 /well in 24-well plates (Nunc).

Two U/ml of Interleukin-2 ala-125 (Amgen) was added on Day 3. The cell density was adjusted to 1×10^6 /ml as needed, and the medium was supplemented with 2 U/ml additional Interleukin-2 to compensate 35 for the increase in volume. Cells were restimulated with peptide-pulsed peripheral blood lymphocytes every 7 days as described below. Interleukin-2 ala-125 (Amgen) was replenished every 3 days.

Cytotoxic T lymphocytes and unstimulated PBLs were frozen (CryoMed) in a mixture of 70% RPMI 1640 (GIBCO), 20% fetal bovine serum (HyClone), and 10% dimethyl sulfoxide (Sigma) and thawed as needed.

EXAMPLE 31

Recovery and Restimulation of Frozen CTL's

45 Cytotoxic T lymphocytes (CTL's) were thawed in a 37° water bath and then resuspended in 35 ml of CTL medium [RPMI 1640 (GIBCO) supplemented with 10% donor or pooled human plasma, 4 mM L-glutamine, 10 mM HEPES, penicillin 100 U/ml and streptomycin 100 μ g/ml (GIBCO)]. The cytotoxic T lymphocytes were then placed at 37°, 5% CO₂ for 1 hour. The cell suspension was centrifuged for 10 minutes at 92 X g. The cells were resuspended at 5×10^5 /ml in CTL medium.

50 The source of stimulator cells for the freshly thawed cytotoxic T lymphocytes was freshly harvested PBL, which had been collected using the Leucoprep method described above. For peptide pulsing, an appropriate number (2×10^5 - 10^7) of PBL were centrifuged, the supernatant was aspirated, and KKAM1 at 200 μ g/ml in RPMI 1640 (GIBCO) plus 10% DMSO (Sigma) was added at the rate of 100 μ l of KKAM1 for every 10^7 cells. The cells were incubated for 1 hour at 37°, 5% CO₂. The peptide-pulsed peripheral blood 55 lymphocytes were irradiated with 2,000 Rads using a ⁶⁰Co source. The cells were washed once in RPMI 1640, centrifuged for 10 minutes at 92 X g, and resuspended in CTL medium at 1×10^5 /ml.

Equal volumes of cytotoxic T lymphocytes and irradiated, peptide-pulsed peripheral blood lymphocytes were mixed together for a final ratio of 1 CTL:2 peptide-pulsed PBL. Interleukin-2 ala-125 (Amgen) was

added at a final concentration of 2 U/ml. The cells were thoroughly mixed together with the Interleukin-2 ala-125 and 1.2 ml was plated into each well of a 48-well plate (CoStar).

The cells were counted and Interleukin-2 ala-125 was replenished every 3 days. This was achieved by pooling all the wells into a centrifuge tube, counting the cells in a hemocytometer counting chamber, adjusting the cells to 1×10^6 /ml with CTL medium, and adding 2 U/ml of Interleukin-2 ala-125. Then 1.5×10^6 cytotoxic T lymphocytes in 1.5 ml of CTL medium with Interleukin-2 ala-125 were plated into each well of a 24-well plate (CoStar). the restimulation process was repeated every seven days, at which time frozen PBL's were then used as the source of stimulators.

10 Example 32

Binding of PEMa to the PE receptor

PEMa was used in a binding/competition assay to compete with PE for the PE receptor on U-2 OS cells. In doing so, PEMa was shown in Figure 6 to protect the cells from the toxic effects of PE. Therefore, replacement of the toxin domain of PE with the Influenza matrix peptide (amino acids 57-68) did not prohibit the binding of this chimeric protein to the FE receptor. This suggests that the ability of PEMa to sensitize target cells for lysis by CTLs specific for the matrix peptide is mediated through PE receptor-mediated uptake and processing.

U-2 cells were grown to a density of 20,000 cells/100 μ l in 960 well plates. Cells were preincubated with PEMa (0,0.1, 1, 10 and 50 μ g in 100 μ l of complete McCoy's 5A medium) for 30 minutes at 37° C, followed by incubation with or without PE(10 ng) for 2 minutes. This represents a 0-, 10-, 100-, 1000-, and 5000-fold excess of PEMa over PE, respectively. Cells were washed with McCoy's medium (3 x 200 μ l), then incubated with [35 S]methionine (2 μ Ci/100 μ l) for an additional 5 hours at 37° C and washed (3 x 200 μ l). Cells were lysed in 10mM EDTA (100 μ l) and aliquots (5 μ l) were spotted onto whatman 3MM filters. Incorporation of radioactivity was assayed by TCA precipitation of the cellular proteins onto the filter papers by immersion into ice-cold TCA (10% w/v) for at least 1 hour. Filters were washed once with 5% TCA and 3 times with ethanol and dried. Radioactivity was determined by liquid scintillation counting. Incorporation of [35 S]methionine into the TCA-precipitable pool of cellular proteins in the absence (open circles) or presence (closed circles) of PE is shown as a function of log excess PEMa. Error bars represent \pm SEM for n=9. Using a one-tailed t-test, incorporation of [35 S]methionine was determined to be significantly lower in the presence of PE than in the absence of FE at 0-, 10-, and 100-fold excesses of PEMa (99.5%, 99.5% and 95% confidence limits, respectively). However, at 1000- and 5000-fold excesses of PEMa, incorporation was not significantly different in the presence or absence of PE.

Following preparation of the protein hybrids of the present invention, a suspension of the protein-hybrids suitable for injection into the host animal must be prepared. Typical suspension vehicles include sterile saline and sterile water for injection. Various agents may be added as preservatives including benzethonium chloride (0.0025%), phenol (0.5%), thiomersal (1:10,000). Strength of the vaccine will be measured as mass of fusion protein which generates a protective response, defined by in vitro/in vivo results, per given host species, a method known to those of ordinary skill in the art.

The suspensions for injection must, of course, be prepared under sterile conditions, in which there is a total absence of living organisms and absolute freedom from biological contamination present in the suspension for injection.

Although water is always the solvent of choice for an injectable preparation, co-solvents that may be additionally present include ethyl alcohol, glycerin, propylene glycol, polyethylene glycol and dimethylacetamide. Buffers may be added, including acidic acid, citric acid or phosphoric acid systems. Antioxidants can include ascorbic acid, BHA, BHT, sodium bisulfite, and sodium metabisulfite. Tonicity can be adjusted with agents such as dextrose, sodium chloride and sodium sulfate.

Aseptic manufacture of vaccines, including their packaging, is conducted according to methods well known to those of ordinary skill in the art, and as described in standard texts on the subject, including Lachman, L., et al., The Theory And Practice of Industrial Pharmacy, Dittert, L., ed, Sprowl's American Pharmacy; and Remington's Pharmaceutical Sciences.

While the invention has been described and illustrated in reference to certain preferred embodiments thereof, those skilled in the art will appreciate that various changes, modifications and substitutions can be made therein without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be limited only by the scope of the claims which follow, and that such claims be interpreted as broadly as is reasonable.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

10

(i) APPLICANT: Liu, Margaret

Oliff, Allen

Donnelly, John

Hawe, Linda

15

Ulmer, Jeffrey

Shi, Xiao-Ping

Friedman, Arthur

20

Montgomery, Donna

(ii) TITLE OF INVENTION: Cellular Immunity
Vaccines From

25

Bacterial Toxin-Antigen Conjugates

(iii) NUMBER OF SEQUENCES: 58

30

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Merck & Co., Inc.

35

(B) STREET: 126 Lincoln Avenue

(C) CITY: Rahway

(D) STATE: New Jersey

(E) COUNTRY: U.S.

40

(F) ZIP: 07065

45

50

55

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0,

Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US
- (B) FILING DATE:
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Grassler, Frank P.
- (B) REGISTRATION NUMBER: 31,164
- (C) REFERENCE/DOCKET NUMBER: 18475

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (908)594-3462
- (B) TELEFAX: (908)594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1294 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15	TCGCGATTGC AGTGGCACTG GCTGGTTTCG CTACCGTAGC GCAGGCCGCG AATTTGGCCG	60
	AAGAAGCTTT CGACCTCTGG AACGAATGCG CCAAAGCCTG CGTGCTCGAC CTCAAGGACG	120
20	GCGTGCGTTC CAGCCGCATG AGCGTCGACC CGGCCATCGC CGACACCAAC GGCCAGGGCG	180
	TGCTGCACTA CTCCATGGTC CTGGAGGGCG GCAACGACGC GCTCAAGCTG GCCATCGACA	240
	ACGCCCTCAG CATCACCAGC GACGGCCTGA CCATCCGCCT CGAAGGCGGC GTCGAGCCGA	300
25	ACAAGCCGGT GCGCTACAGC TACACGCGCC AGGCGCGCGG CAGTTGGTCG CTGAACTGGC	360
	TGGTACCGAT CGGCCACGAG AAGCCCTCGA ACATCAAGGT GTTCATCCAC GAACTGAACG	420
	CCGGCAACCA GCTCAGCCAC ATGTCGCCGA TCTACACCAT CGAGATGGGC GACGAGTTGC	480
30	TGGCGAAGCT GGCGCGCGAT GCCACCTTCT TCGTCAGGGC GCACGAGAGC AACGAGATGC	540
	AGCCGACGCT CGCCATCAGC CATGCCGGGG TCAGCGTGGT CATGGCCCAG ACCCAGCCGC	600
35	GCCGGGAAAA GCGCTGGAGC GAATGGGCCA GCGGCAAGGT GTTGTGCCTG CTCGACCCGC	660
	TGGACGGGGT CTACAACTAC CTCGCCCAGC AACGCTGCAA CCTCGACGAT ACCTGGGAAG	720
40	GCAAGATCTA CCGGTGCTC GCCGGCAACC CGGCGAAGCA TGACCTGGAC ATCAAACCCA	780
	CGGTCATCAG TCATCGCCTG CACTTTCCCG AGGGCGGCAG CCTGGCCGCG CTGACCGCGC	840
	ACCAGGCTTG CCACCTGCCG CTGGAGACTT TCACCCGTCA TCGCCAGCCG CGCGGCTGGG	900
45	AACAACTGGA GCAGTGCGGC TATCCGGTGC AGCGGCTGGT CGCCCTCTAC CTGGCGGCGC	960
	GGCTGTCTGT GAACCAGGTC GACCAGGTGA TCCGCAACGC CCTGGCCAGC CCCGGCAGCG	1020

GCGGCGACCT GGGCGAAGCG ATCCGCGAGC AGCCGGAGCA GGCCCGTCTG GCCCTGACCC 1080
 TGGCCGCCGC CGAGAGCGAG CGCTTCGTCC GGCAGGGCAC CGGCAACGAC GAGGCCGGCG 1140
 5 CGGCCAACGC CGACGTGGTG AGCCTGACCT GCCCGGTCGC CGCCGGTGAA TGC CGGGGCC 1200
 CGGCGGACAG CGGCGACGCC CTGCTGGAGC GCAACTATCC CACTGGCGCG GAGTTCCTCG 1260
 10 GCGACGGCGG CGACGTCAGC TTCAGCACCC GCGG 1294

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 759 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 ATGAGTCTTC TAACCGAGGT CGAAACGTAC GTTCTCTCTA TCATCCCGTC AGGCCCCCTC 60
 AAAGCCGAGA TCGCACAGAG ACTTGAAGAT GTCTTTGCAG GGAAGAACAC CGATCTTGAG 120
 30 GTTCTCATGG AATGGCTAAA GACAAGACCA ATCCTGTCAC CTCTGACTAA GGGGATTTTA 180
 GGATTTGTGT TCACGCTCAC CGTGCCCACT GAGCGAGGAC TGCAGCGTAG ACGCTTTGTC 240
 CAAAATGCCC TTAATGGGAA CGGGGATCCA AATAACATGG ACAAAGCAGT TAAACTGTAT 300
 35 AGGAAGCTCA AGAGGGAGAT AACATTCCAT GGGGCCAAAG AAATCTCACT CAGTTATTCT 360
 GCTGGTGAC TTGCCAGTTG TATGGGCCTC ATATAACA GAATGGGGGC TGTGACCACT 420
 40 GAAGTGGCAT TTGGCCTGGT ATGTGCAACC TGTGAACAGA TTGCTGACTC CCAGCATCGG 480
 TCTCATAGGC AAATGGTGAC AACAACCAAC CCACTAATCA GACATGAGAA CAGAATGGTT 540
 TTAGCCAGCA CTACAGCTAA GGCTATGGAG CAAATGGCTG GATCGAGTGA GCAAGCAGCA 600
 45 GAGGCCATGG AGGTTGCTAG TCAGGCTAGG CAAATGGTGC AAGCGATGAG AACCATTGGG 660

ACTCATCCTA GCTCCAGTGC TGGTCTGAAA AATGATCTTC TTGAAAATTT GCAGGCCTAT 720

CAGAAACGAA TGGGGGTGCA GATGCAACGG TTCAAGTGA 759

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 253 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro
1 5 10 15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
20 25 30

Ala Gly Lys Asn Thr Asp Leu Glu Val Leu Met Glu Trp Leu Lys Thr
35 40 45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
50 55 60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65 70 75 80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala
85 90 95

Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
100 105 110

Lys Glu Ile Ser Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
115 120 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
130 135 140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg
145 150 155 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
165 170 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
180 185 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
195 200 205

Ala Arg Gln Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser
210 215 220

Ser Ser Ala Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225 230 235 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys Xaa
245 250

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATACCCGCGG CAGTCTTCTA ACCGAGGTCG

30

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCCACGTCT ACGTTGCCAA GTTCACTCTC GAGATA

36

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCGAGAATT CATGGCCGAG GAAGCTT

27

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1998 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGCCGAAG AAGCTTTCGA CCTCTGGAAC GAATGCGCCA AAGCCTGCGT GCTCGACCTC 60
AAGGACGGCG TCGTTCAG CCGCATGAGC GTCGACCCGG CCATCGCCGA CACCAACGGC 120
CAGGGCGTGC TGCACTACTC CATGGTCCTG GAGGGCGGCA ACGACGCGCT CAAGCTGGCC 180
ATCGACAACG CCCTCAGCAT CACCAGCGAC GGCCTGACCA TCCGCCTCGA AGGCGGCGTC 240
GAGCCGAACA AGCCGGTGCG CTACAGCTAC ACGCGCCAGG CGCGCGGCAG TTGGTCGCTG 300
AACTGGCTGG TACCGATCGG CCACGAGAAG CCCTCGAACA TCAAGGTGTT CATCCACGAA 360
CTGAACGCCG GCAACCAGCT CAGCCACATG TCGCCGATCT ACACCATCGA GATGGGCGAC 420
GAGTTGCTGG CGAAGCTGGC GCGCGATGCC ACCTTCTTCG TCAGGGCGCA CGAGAGCAAC 480
GAGATGCAGC CGACGCTCGC CATCAGCCAT GCCGGGGTCA GCGTGGTCAT GGCCAGACC 540

CAGCCGCGCC GGGAAAAGCG CTGGAGCGAA TGGGCCAGCG GCAAGGTGTT GTGCCTGCTC	600
GACCCGCTGG ACGGGGTCTA CAACTACCTC GCCCAGCAAC GCTGCAACCT CGACGATACC	660
TGGGAAGGCA AGATCTACCG GGTGCTCGCC GGCAACCCGG CGAAGCATGA CCTGGACATC	720
AAACCCACGG TCATCAGTCA TCGCCTGCAC TTTCCCGAGG GCGGCAGCCT GGCCGCGCTG	780
ACCGCGCACC AGGCTTGCCA CCTGCCGCTG GAGACTTTCA CCCGTCATCG CCAGCCGCGC	840
GGCTGGGAAC AACTGGAGCA GTGCGGTAT CCGGTGCAGC GGCTGGTCGC CCTCTACCTG	900
GCGGCGCGGC TGTCGTGGAA CCAGGTGCAC CAGGTGATCC GCAACGCCCT GGCCAGCCCC	960
GGCAGCGGCG GCGACCTGGG CGAAGCGATC CGCGAGCAGC CGGAGCAGGC CCGTCTGGCC	1020
CTGACCCTGG CCGCCGCCGA GAGCGAGCGC TTCGTCCGGC AGGGCACC GG CAACGACGAG	1080
GCCGGCGCGG CCAACGCCGA CGTGGTGAGC CTGACCTGCC CGGTCGCCG CCGTGAATGC	1140
GCGGGCCCGG CGGACAGCGG CGACGCCCTG CTGGAGCGCA ACTATCCCAC TGGCGCGGAG	1200
TTCTCTGGCG ACGGCGGCGA CGTCAGCTT AGCACCCGCG GCAGTCTTCT AACCGAGGTC	1260
GAAACGTACG TTCTCTCTAT CATCCCGTCA GGCCCCCTCA AAGCCGAGAT CGCACAGAGA	1320
CTTGAAGATG TCTTTCAGG GAAGAACACC GATCTTGAGG TTCTCATGGA ATGGCTAAAG	1380
ACAAGACCAA TCCTGTCACC TCTGACTAAG GGGATTTTAG GATTTGTGTT CACGCTCACC	1440
GTGCCCAGTG AGCGAGGACT GCAGCGTAGA CGCTTTGTCC AAAATGCCCT TAATGGGAAC	1500
GGGGATCCAA ATAACATGGA CAAAGCAGTT AAAGTGTATA GGAAGCTCAA GAGGGAGATA	1560
ACATTCCATG GGGCCAAAGA AATCTCACTC AGTTATTCTG CTGGTGCCTG TGCCAGTTGT	1620
ATGGGCCTCA TATACAACAG GATGGGGGCT GTGACCACTG AAGTGGCATT TGGCCTGGTA	1680
TGTGCAACCT GTGAACAGAT TGCTGACTCC CAGCATCGGT CTCATAGGCA AATGGTGACA	1740
ACAACCAACC CACTAATCAG ACATGAGAAC AGAATGGTTT TAGCCAGCAC TACAGCTAAG	1800
GCTATGGAGC AAATGGCTGG ATCGAGTGAG CAAGCAGCAG AGGCCATGGA GGTGCTAGT	1860
CAGGCTAGGC AAATGGTGCA AGCGATGAGA ACCATTGGGA CTCATCCTAG CTCCAGTGCT	1920
GGTCTGAAAA ATGATCTTCT TGAAAATTG CAGGCCTATC AGAAACGAAT GGGGGTGCAG	1980
ATGCAACGGT TCAAGTGA	1998

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 666 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys
 1 5 10 15

Val Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp
 20 25 30

Pro Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met
 35 40 45

Val Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala
 50 55 60

Leu Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val
 65 70 75 80

Glu Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly
 85 90 95

Ser Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser
 100 105 110

Asn Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser
 115 120 125

His Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala
 130 135 140

Lys Leu Ala Arg-Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn
 145 150 155 160

Glu Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val
 165 170 175

Met Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala
 180 185 190

EP 0 532 090 A2

Ser Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn
 195 200 205
 Tyr Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys
 5 210 215 220
 Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile
 225 230 235 240
 Lys Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser
 10 245 250 255
 Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr
 15 260 265 270
 Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys
 275 280 285
 Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu
 20 290 295 300
 Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro
 305 310 315 320
 Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln
 25 325 330 335
 Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val
 340 345 350
 Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val
 30 355 360 365
 Val Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala
 35 370 375 380
 Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu
 385 390 395 400
 Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Ser Leu
 40 405 410 415
 Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro Ser Gly Pro
 420 425 430
 Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe Ala Gly Lys
 45 435 440 445

Asn Thr Asp Leu Glu Val Leu Met Glu Trp Leu Lys Thr Arg Pro Ile
450 455 460

5 Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe Thr Leu Thr
465 470 475 480

Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val Gln Asn Ala
485 490 495

10 Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala Val Lys Leu
500 505 510

15 Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala Lys Glu Ile
515 520 525

Ser Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met Gly Leu Ile
530 535 540

20 Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe Gly Leu Val
545 550 555 560

Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg Ser His Arg
565 570 575

25 Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu Asn Arg Met
580 585 590

30 Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met Ala Gly Ser
595 600 605

Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln Ala Arg Gln
610 615 620

35 Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser Ser Ser Ala
625 630 635 640

Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr Gln Lys Arg
645 650 655

40 Met Gly Val Gln Met Gln Arg Phe Lys Xaa
660 665

(2) INFORMATION FOR SEQ ID NO:9:

- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 52 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic) .

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTAGAAATAA TTTTGTTTAA CITTAAGAAG GAGATATACA TATGGCCGAA GA

52

10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATACCCGCGG CAAGGGGATT TTAGGATTTG TG

32

25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATAGAGCTCT CACACGGTGA GCGTGAACAC AAATCC

36

40

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 52 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

45

50

55

(ii) MOLECULE TYPE: DNA (genomic)

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCGCGGCAAG GGGATTTTAG GATTTGTGTT CACGCTCACC GTGTGAGAGC TC

52

10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 52 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTAGAAATAA TTTTGTTTAA CTTAAGAAG GAGATATACA TATGGCCGAA GA

52

25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1281 base pairs

30

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

40

ATGGCCGAGG AAGCTTTTGA CCTCTGGAAC GAATGCGCCA AAGCCTGCGT GCTCGACCTC

60

AAGGACGGCG TGC GTTCCAG CCGCATGAGC GTCGACCCGG CCATCGCCGA CACCAACGGC

120

CAGGGCGTGC TGCACTACTC CATGGTCCTG GAGGGCGGCA ACGACGCGCT CAAGCTGGCC

180

45

ATCGACAACG CCCTCAGCAT CACCAGCGAC GGCCTGACCA TCCGCCTCGA AGGCGGCGTC

240

GAGCCGAACA AGCCGGTGCG CTACAGCTAC ACGCGCCAGG CGCGCGGCAG TTGGTCGCTG

300

50

55

AACTGGCTGG TACCGATCGG CCACGAGAAG CCCTCGAACA TCAAGGTGTT CATCCACGAA 360
 CTGAACGCCG GCAACCAGCT CAGCCACATG TCGCCGATCT ACACCATCGA GATGGGCGAC 420
 5 GAGTTGCTGG CGAAGCTGGC GCGCGATGCC ACCTTCTTCG TCAGGGCGCA CGAGAGCAAC 480
 GAGATGCAGC CGACGCTCGC CATCAGCCAT GCCGGGGTCA GCGTGGTCAT GGCCAGACC 540
 10 CAGCCGCGCC GGGAAAAGCG CTGGAGCGAA TGGGCCAGCG GCAAGGTGTT GTGCCTGCTC 600
 GACCCGCTGG ACGGGGTCTA CAACTACCTC GCCCAGCAAC GCTGCAACCT CGACGATACC 660
 TGGGAAGGCA AGATCTACCG GGTGCTCGCC GGCAACCCGG CGAAGCATGA CCTGGACATC 720
 15 AAACCCACGG TCATCAGTCA TCGCCTGCAC TTTCCCAGG GCGGCAGCCT GGCCGCGCTG 780
 ACCGCGCACC AGGCTTGCCA CCTGCCGCTG GAGACTTTCA CCCGTCATCG CCAGCCGCGC 840
 20 GGCTGGGAAC AACTGGAGCA GTGCGGTAT CCGGTGCAGC GGCTGGTCGC CCTCTACCTG 900
 GCGGCGCGGC TGTCGTGGAA CCAGGTCGAC CAGGTGATCC GCAACGCCCT GGCCAGCCCC 960
 GGCAGCGGCG GCGACCTGGG CGAAGCGATC CGCGAGCAGC CGGAGCAGGC CCGTCTGGCC 1020
 25 CTGACCCTGG CCGCCGCCGA GAGCGAGCGC TTCGTCCGGC AGGGCACC GG CAACGACGAG 1080
 GCCGGCGCGG CCAACGCCGA CGTGGTGAGC CTGACCTGCC CGGTCGCCGC CGGTGAATGC 1140
 30 GCGGGCCCGG CGGACAGCGG CGACGCCCTG CTGGAGCGCA ACTATCCCAC TGGCGCGGAG 1200
 TTCCTCGGCG ACGGCGGCGA CGTCAGCTTC AGCACCCGCG GCAAGGGGAT TTAGGATTT 1260
 GTGTTACGC TCACCGTGTG A 1281

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 427 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

5	Met	Ala	Glu	Glu	Ala	Phe	Asp	Leu	Trp	Asn	Glu	Cys	Ala	Lys	Ala	Cys	1	5	10	15
	Val	Leu	Asp	Leu	Lys	Asp	Gly	Val	Arg	Ser	Ser	Arg	Met	Ser	Val	Asp	20	25	30	
10	Pro	Ala	Ile	Ala	Asp	Thr	Asn	Gly	Gln	Gly	Val	Leu	His	Tyr	Ser	Met	35	40	45	
	Val	Leu	Glu	Gly	Gly	Asn	Asp	Ala	Leu	Lys	Leu	Ala	Ile	Asp	Asn	Ala	50	55	60	
15	Leu	Ser	Ile	Thr	Ser	Asp	Gly	Leu	Thr	Ile	Arg	Leu	Glu	Gly	Gly	Val	65	70	75	80
	Glu	Pro	Asn	Lys	Pro	Val	Arg	Tyr	Ser	Tyr	Thr	Arg	Gln	Ala	Arg	Gly	85	90	95	
20	Ser	Trp	Ser	Leu	Asn	Trp	Leu	Val	Pro	Ile	Gly	His	Glu	Lys	Pro	Ser	100	105	110	
	Asn	Ile	Lys	Val	Phe	Ile	His	Glu	Leu	Asn	Ala	Gly	Asn	Gln	Leu	Ser	115	120	125	
25	His	Met	Ser	Pro	Ile	Tyr	Thr	Ile	Glu	Met	Gly	Asp	Glu	Leu	Leu	Ala	130	135	140	
30	Lys	Leu	Ala	Arg	Asp	Ala	Thr	Phe	Phe	Val	Arg	Ala	His	Glu	Ser	Asn	145	150	155	160
	Glu	Met	Gln	Pro	Thr	Leu	Ala	Ile	Ser	His	Ala	Gly	Val	Ser	Val	Val	165	170	175	
35	Met	Ala	Gln	Thr	Gln	Pro	Arg	Arg	Glu	Lys	Arg	Trp	Ser	Glu	Trp	Ala	180	185	190	
40	Ser	Gly	Lys	Val	Leu	Cys	Leu	Leu	Asp	Pro	Leu	Asp	Gly	Val	Tyr	Asn	195	200	205	
	Tyr	Leu	Ala	Gln	Gln	Arg	Cys	Asn	Leu	Asp	Asp	Thr	Trp	Glu	Gly	Lys	210	215	220	
45	Ile	Tyr	Arg	Val	Leu	Ala	Gly	Asn	Pro	Ala	Lys	His	Asp	Leu	Asp	Ile	225	230	235	240
50	Lys	Pro	Thr	Val	Ile	Ser	His	Arg	Leu	His	Phe	Pro	Glu	Gly	Gly	Ser	245	250	255	

55

Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr
260 265 270

Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys
275 280 285

Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu
290 295 300

Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro
305 310 315 320

Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln
325 330 335

Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val
340 345 350

Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val
355 360 365

Val Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala
370 375 380

Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu
385 390 395 400

Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Lys Gly
405 410 415

Ile Leu Gly Phe Val Phe Thr Leu Thr Val Xaa
420 425

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:--DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGCTGATAAT AGAGCTCG

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1245 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

15	ATGGCCGAGG AAGCTTTCGA CCTCTGGAAC GAATGCGCCA AAGCCTGCGT GCTCGACCTC	60
	AAGGACGGCG TGC GTTCCAG CCGCATGAGC GTCGACCCGG CCATCGCCGA CACCAACGGC	120
20	CAGGGCGTGC TGCACTACTC CATGGTCTTG GAGGGCGGCA ACGACGCGCT CAAGCTGGCC	180
	ATCGACAACG CCCTCAGCAT CACCAGCGAC GGCCTGACCA TCCGCCTCGA AGGCGGCGTC	240
	GAGCCGAACA AGCCGGTGCG CTACAGCTAC ACGCGCCAGG CGCGCGGCG TGGTCTGCTG	300
25	AACTGGCTGG TACCGATCGG CCACGAGAAG CCCTCGAACA TCAAGGTGTT CATCCACGAA	360
	CTGAACGCCG GCAACCAGCT CAGCCACATG TCGCCGATCT ACACCATCGA GATGGGCGAC	420
30	GAGTTGCTGG CGAAGCTGGC GCGCGATGCC ACCTTCTTCG TCAGGGCGCA CGAGAGCAAC	480
	GAGATGCAGC CGACGCTCGC CATCAGCCAT GCCGGGGTCA GCGTGGTCAT GGCCAGACC	540
	CAGCCGCGCC GGGAAAAGCG CTGGAGCGAA TGGGCCAGCG GCAAGGTGTT GTGCCTGCTC	600
35	GACCCGCTGG ACGGGGTCTA CAACTACCTC GCCCAGCAAC GCTGCAACCT CGACGATACC	660
	TGGGAAGGCA AGATCTACCG GGTGCTCGCC GGCAACCCGG CGAAGCATGA CCTGGACATC	720
40	AAACCCACGG TCATCAGTCA TCGCTGCAC TTTCCCGAGG GCGGCAGCCT GGCCGCGCTG	780
	ACCGCGCACC AGGCTTGCCA CCTGCCGCTG GAGACTTTCA CCCGTCATCG CCAGCCGCGC	840
	GGCTGGGAAC AACTGGAGCA GTGCGGCTAT CCGGTGCAGC GGCTGGTCGC CCTCTACCTG	900
45	GCGGCGCGGC TGTCGTGGAA CCAGGTCGAC CAGGTGATCC GCAACGCCCT GGCCAGCCCC	960
	GGCAGCGGCG GCGACCTGGG CGAAGCGATC CGCGAGCAGC CGGAGCAGGC CCGTCTGGCC	1020

CTGACCCTGG CCGCCGCCGA GAGCGAGCGC TTCGTCCGGC AGGGCACCGG CAACGACGAG 1080
 GCCGGCGCGG CCAACGCCGA CGTGGTGAGC CTGACCTGCC CGGTCGCCGC CGGTGAATGC 1140
 GCGGGCCCCG CGGACAGCGG CGACGCCCTG CTGGAGCGCA ACTATCCCAC TGGCGCGGAG 1200
 TTCCTCGGCG ACGGCGGCGA CGTCAGCTTC AGCACCCGCG GCTGA 1245

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 415 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys
 1 5 10 15
 Val Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp
 20 25 30
 Pro Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met
 35 40 45
 Val Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala
 50 55 60
 Leu Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val
 65 70 75 80
 Glu Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly
 85 90 95
 Ser Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser
 100 105 110
 Asn Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser
 115 120 125
 His Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala
 130 135 140

Lys Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn
 145 150 155 160
 5 Glu Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val
 165 170 175
 Met Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala
 180 185 190
 10 Ser Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn
 195 200 205
 Tyr Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys
 210 215 220
 15 Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile
 225 230 235 240
 20 Lys Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser
 245 250 255
 Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr
 260 265 270
 25 Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys
 275 280 285
 Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu
 290 295 300
 30 Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro
 305 310 315 320
 Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln
 325 330 335
 Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val
 340 345 350
 40 Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val
 355 360 365
 Val Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala
 370 375 380
 45 Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu
 385 390 395 400
 50
 55

Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Xaa
405 410 415

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCGAGCCGCC ACCATGGCCG AGGAA

25

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 46 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GACCCGCTAG CACCCGGGAA ACCGCCGCGC GAGGACCTGA AGTAAG

46

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1956 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

	ATGCACCTGA TACCCCATTTG GATCCCCCTG GTCGCCAGCC TCGGCCTGCT CGCCGGCGGC	60
5	TCGTCCGCGT CCGCCGCCGA GGAAGCTTTC GACCTCTGGA ACGAATGCGC CAAAGCCTGC	120
	GTGCTCGACC TCAAGGACGG CGTGCGTTCC AGCCGCATGA GCGTCGACCC GGCCATCGCC	180
10	GACACCAACG GCCAGGGCGT GCTGCACTAC TCCATGGTCC TGGAGGGCGG CAACGACGCG	240
	CTCAAGCTGG CCATCGACAA CGCCCTCAGC ATCACCAGCG ACGGCCTGAC CATCCGCCTC	300
	GAAGGCGGCG TCGAGCCGAA CAAGCCGGTG CGTACAGCT ACACGCGCCA GGCGCGCGGC	360
15	AGTTGGTCGC TGAAGTGGCT GGTACCGATC GGCCACGAGA AGCCCTCGAA CATCAAGGTG	420
	TTCATCCACG AACTGAACGC CGGCAACCAG CTCAGCCACA TGTCGCCGAT CTACACCATC	480
20	GAGATGGGCG ACGAGTTGCT GGCGAAGCTG GCGCGCGATG CCACCTTCTT CGTCAGGGCG	540
	CACGAGAGCA ACGAGATGCA GCCGACGCTC GCCATCAGCC ATGCCGGGGT CAGCGTGGTC	600
	ATGGCCCAGA CCCAGCCGCG CCGGGAAGAG CGCTGGAGCG AATGGGCCAG CGGCAAGGTG	660
25	TTGTGCCTGC TCGACCCGCT GGACGGGGTC TACAACTACC TCGCCCAGCA ACGCTGCAAC	720
	CTCGACGATA CCTGGGAAGG CAAGATCTAC CGGGTGCTCG CCGGCAACCC GGCGAAGCAT	780
30	GACCTGGACA TCAAACCCAC GGTCATCAGT CATCGCCTGC ACTTTCCCGA GGGCGGCAGC	840
	CTGGCCGCGC TGACCGCGCA CCAGGCTTGC CACCTGCCGC TGGAGACTTT CACCCGTCAT	900
	CGCCAGCCGC GCGGCTGGGA ACAACTGGAG CAGTGCGGCT ATCCGGTGCA GCGGCTGGTC	960
35	GCCCTCTACC TGGCGGCGCG GCTGTCTGTG AACCAGGTG ACCAGGTGAT CCGCAACGCC	1020
	CTGGCCAGCC CCGGCAGCGG CGGCGACCTG GCGGAAGCGA TCCGCGAGCA GCCGGAGCAG	1080
40	GCCCGTCTGG CCCTGACCTT GGCCGCCGCC GAGAGCGAGC GCTTCGTCCG GCAGGGCACC	1140
	GGCAACGACG AGGCCGCGC GGCCAACGCC GACGTGGTGA GCCTGACCTG CCCGGTCGCC	1200
	GCCGGTGAAT GCGCGGGCCC GCGGACAGC GCGCAGCCCC TGCTGGAGCG CAACTATCCC	1260
45	ACTGGCGCGG AGTTCCTCGG CGACGGCGGC GACGTCAGCT TCAGCACCCG CGGCACGCAG	1320
	AACTGGACGG TGGAGCGGCT GCTCCAGGCG CACCGCCAAC TGGAGGAGCG CGGCTATGTG	1380

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TTCGTCGGCT ACCACGGCAC CTTCTCGAA GCGGCGCAAA GCATCGTCTT CGGCGGGGTG 1440
 CGCGCGCGCA GCCAGGACCT CGACGCGATC TGGCGCGGTT TCTATATCGC CGGCGATCCG 1500
 GCGCTGGCCT ACGGCTACGC CCAGGACCAG GAACCCGACG CACGCGGCCG GATCCGCAAC 1560
 GGTGCCCTGC TCGGGGTCTA TGTGCCGCGC TCGAGCCTGC CGGGCTTCTA CCGCACCAGC 1620
 CTGACCCTGG CCGCGCCGGA GCGGCGGGC GAGGTGGAAC GGCTGATCGG CCATCCGCTG 1680
 CCGCTGCGCC TGGACGCCAT CACCGGCCCC GAGGAGGAAG GCGGGCGCCT GGAGACCATT 1740
 CTCGGCTGGC CGCTGGCCGA GCGCACCCTG GTGATTCCCT CGGCGATCCC CACCGACCCG 1800
 CGCAACGTCG GCGGCGACCT CGACCCGTCC AGCATCCCCG ACAAGGAACA GCGGATCAGC 1860
 GCCCTGCCGG ACTAGCCAG CCAGCCCGGC AAACCGCCGC GCGAGGACCC GCTAGCACCC 1920
 GGGAAACCGC CGCGCGAGGA CCTGAAGTAA GAATTC 1956

(2) INFORMATION FOR SEQ ID NO:22:

25
 30

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 652 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

35
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Met His Leu Ile Pro His Trp Ile Pro Leu Val Ala Ser Leu Gly Leu
 1 5 10 15
 Leu Ala Gly Gly Ser Ser Ala Ser Ala Ala Glu Glu Ala Phe Asp Leu
 20 25 30
 Trp Asn Glu Cys Ala Lys Ala Cys Val Leu Asp Leu Lys Asp Gly Val
 35 40 45
 Arg Ser Ser Arg Met Ser Val Asp Pro Ala Ile Ala Asp Thr Asn Gly
 50 55 60
 Gln Gly Val Leu His Tyr Ser Met Val Leu Glu Gly Gly Asn Asp Ala
 65 70 75 80

Leu Lys Leu Ala Ile Asp Asn Ala Leu Ser Ile Thr Ser Asp Gly Leu
 85 90 95
 Thr Ile Arg Leu Glu Gly Gly Val Glu Pro Asn Lys Pro Val Arg Tyr
 5 100 105 110
 Ser Tyr Thr Arg Gln Ala Arg Gly Ser Trp Ser Leu Asn Trp Leu Val
 115 120 125
 Pro Ile Gly His Glu Lys Pro Ser Asn Ile Lys Val Phe Ile His Glu
 10 130 135 140
 Leu Asn Ala Gly Asn Gln Leu Ser His Met Ser Pro Ile Tyr Thr Ile
 145 150 155 160
 15 Glu Met Gly Asp Glu Leu Leu Ala Lys Leu Ala Arg Asp Ala Thr Phe
 165 170 175
 Phe Val Arg Ala His Glu Ser Asn Glu Met Gln Pro Thr Leu Ala Ile
 20 180 185 190
 Ser His Ala Gly Val Ser Val Val Met Ala Gln Thr Gln Pro Arg Arg
 195 200 205
 25 Glu Lys Arg Trp Ser Glu Trp Ala Ser Gly Lys Val Leu Cys Leu Leu
 210 215 220
 Asp Pro Leu Asp Gly Val Tyr Asn Tyr Leu Ala Gln Gln Arg Cys Asn
 225 230 235 240
 30 Leu Asp Asp Thr Trp Glu Gly Lys Ile Tyr Arg Val Leu Ala Gly Asn
 245 250 255
 Pro Ala Lys His Asp Leu Asp Ile Lys Pro Thr Val Ile Ser His Arg
 35 260 265 270
 Leu His Phe Pro Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
 275 280 285
 40 Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
 290 295 300
 Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
 305 310 315 320
 45 Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
 325 330 335
 50
 55

Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
 340 345 350
 5 Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
 355 360 365
 Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
 370 375 380
 10 Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
 385 390 395 400
 Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
 405 410 415
 15 Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
 420 425 430
 Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
 435 440 445
 Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
 450 455 460
 25 His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
 465 470 475 480
 Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
 485 490 495
 30 Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
 500 505 510
 Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
 515 520 525
 35 Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
 530 535 540
 40 Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
 545 550 555 560
 Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
 565 570 575
 45 Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
 580 585 590
 50
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Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
595 600 605

5 Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
610 615 620

Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Pro Leu Ala Pro
625 630 635 640

10 Gly Lys Pro Pro Arg Glu Asp Leu Lys Xaa Glu Phe
645 650

(2) INFORMATION FOR SEQ ID NO:23:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCGGGCTGAC TAAGGGGATT TTAGGATTTG TGTTACGCT CACCGTGC 48

(2) INFORMATION FOR SEQ ID NO:24:

30 (i) SEQUENCE CHARACTERISTICS:
/ (A) LENGTH: 2004 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATGCACCTGA TACCCATTG GATCCCCCTG GTCGCCAGCC TCGGCCTGCT CGCCGGCGGC 60

45 TCGTCCGCGT CCGCCGCCGA GGAAGCTTTC GACCTCTGGA ACGAATGCGC CAAAGCCTGC 120

GTGCTCGACC TCAAGGACGG CGTGC GTTCC AGCCGCATGA GCGTCGACCC GGCCATCGCC 180

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	GACACCAACG GCCAGGGCGT GCTGCACTAC TCCATGGTCC TGGAGGGCGG CAACGACGCG	240
	CTCAAGCTGG CCATCGACAA CGCCCTCAGC ATCACCAGCG ACGGCCTGAC CATCCGCCTC	300
5	GAAGGCGGCG TCGAGCCGAA CAAGCCGGTG CGCTACAGCT ACACGCGCCA GGCGCGCGGC	360
	AGTTGGTCGC TGAAGTGGCT GGTACCGATC GGCCACGAGA AGCCCTCGAA CATCAAGGTG	420
10	TTCATCCACG AACTGAACGC CGGCAACCAG CTCAGCCACA TGTCGCCGAT CTACACCATC	480
	GAGATGGGCG ACGAGTTGCT GGCGAAGCTG GCGCGCGATG CCACCTTCTT CGTCAGGGCG	540
	CACGAGAGCA ACGAGATGCA GCCGACGCTC GCCATCAGCC ATGCCGGGGT CAGCGTGGTC	600
15	ATGGCCCAGA CCCAGCCGCG CCGGGAAAAG CGCTGGAGCG AATGGGCCAG CGGCAAGGTG	660
	TTGTGCCTGC TCGACCCGCT GGACGGGGTC TACAACTACC TCGCCCAGCA ACGCTGCAAC	720
20	CTCGACGATA CCTGGGAAGG CAAGATCTAC CGGGTGCTCG CCGGCAACCC GGCGAAGCAT	780
	GACCTGGACA TCAAACCCAC GGTCATCAGT CATCGCCTGC ACTTTCCCGA GGGCGGCAGC	840
	CTGGCCGCGC TGACCGCGCA CCAGGCTTGC CACCTGCCGC TGGAGACTTT CACCCGTCAT	900
25	CGCCAGCCGC GCGGCTGGGA ACAACTGGAG CAGTGGGCT ATCCGGTGCA GCGGCTGGTC	960
	GCCCTCTACC TGGCGGCGCG GCTGTCGTGG AACCAGGTG ACCAGGTGAT CCGCAACGCC	1020
30	CTGGCCAGCC CCGGCAGCGG CGGCGACCTG GGCGAAGCGA TCCGCGAGCA GCCGGAGCAG	1080
	GCCCGTCTGG CCCTGACCCT GGCCGCCGCC GAGAGCGAGC GCTTCGTCCG GCAGGGCACC	1140
	GGCAACGACG AGGCCGGCGC GGCCAACGCC GACGTGGTGA GCCTGACCTG CCCGGTCGCC	1200
35	GCCGGTGAAT GCGCGGGCCC GCGGACAGC GGCGACGCC TGCTGGAGCG CAACTATCCC	1260
	ACTGGCGCGG AGTTCCTCGG CGACGGCGGC GACGTCAGCT TCAGCACCCG CGGCACGCAG	1320
40	AACTGGACGG TGGAGCGGCT GCTCCAGGCG CACCGCCAAC TGGAGGAGCG CGGCTATGTG	1380
	TTCGTCGGCT ACCACGGCAC CTTCTCGAA GCGGCGCAAA GCATCGTCTT CGGCGGGGTG	1440
	CGCGCGCGCA GCCAGGACCT CGACGCGATC TGGCGGGTT TCTATATCGC CGGCGATCCG	1500
45	GCGCTGGCCT ACGGCTACGC CCAGGACCAG GAACCCGACG CACGCGGCCG GATCCGCAAC	1560
	GGTGCCCTGC TCGGGGTCTA TGTGCCGCGC TCGAGCCTGC CGGGCTTCTA CCGCACCAGC	1620
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CTGACCCTGG CCGCGCCGGA GCGGCGGGC GAGGTCGAAC GGCTGATCGG CCATCCGCTG 1680
 CCGCTGCGCC TGGACGCCAT CACCGGCCCC GAGGAGGAAG GCGGGCGCCT GGAGACCATT 1740
 CTCGGCTGGC CGCTGGCCGA GCGCACCGTG GTGATTCCCT CGGCGATCCC CACCGACCCG 1800
 CGCAACGTCG GCGGCGACCT CGACCCGTCC AGCATCCCCG ACAAGGAACA GGCGATCAGC 1860
 GCCCTGCCGG ACTACGCCAG CCAGCCCGGC AAACCGCCGC GCGAGGACCC GCTAGCACCC 1920
 GGGCTGACTA AGGGGATTTT AGGATTGTG TTCACGCTCA CCGTGCCCGG GAAACCGCCG 1980
 CGCGAGGACC TGAAGTAAGA ATTC 2004

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 668 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met His Leu Ile Pro His Trp Ile Pro Leu Val Ala Ser Leu Gly Leu
 1 5 10 15
 Leu Ala Gly Gly Ser Ser Ala Ser Ala Ala Glu Glu Ala Phe Asp Leu
 20 25 30
 Trp Asn Glu Cys Ala Lys Ala Cys Val Leu Asp Leu Lys Asp Gly Val
 35 40 45
 Arg Ser Ser Arg Met Ser Val Asp Pro Ala Ile Ala Asp Thr Asn Gly
 50 55 60
 Gln Gly Val Leu His Tyr Ser Met Val Leu Glu Gly Gly Asn Asp Ala
 65 70 75 80
 Leu Lys Leu Ala Ile Asp Asn Ala Leu Ser Ile Thr Ser Asp Gly Leu
 85 90 95
 Thr Ile Arg Leu Glu Gly Gly Val Glu Pro Asn Lys Pro Val Arg Tyr
 100 105 110

Ser Tyr Thr Arg Gln Ala Arg Gly Ser Trp Ser Leu Asn Trp Leu Val
 115 120 125
 5 Pro Ile Gly His Glu Lys Pro Ser Asn Ile Lys Val Phe Ile His Glu
 130 135 140
 Leu Asn Ala Gly Asn Gln Leu Ser His Met Ser Pro Ile Tyr Thr Ile
 145 150 155 160
 10 Glu Met Gly Asp Glu Leu Leu Ala Lys Leu Ala Arg Asp Ala Thr Phe
 165 170 175
 Phe Val Arg Ala His Glu Ser Asn Glu Met Gln Pro Thr Leu Ala Ile
 180 185 190
 15 Ser His Ala Gly Val Ser Val Val Met Ala Gln Thr Gln Pro Arg Arg
 195 200 205
 Glu Lys Arg Trp Ser Glu Trp Ala Ser Gly Lys Val Leu Cys Leu Leu
 210 215 220
 Asp Pro Leu Asp Gly Val Tyr Asn Tyr Leu Ala Gln Gln Arg Cys Asn
 225 230 235 240
 25 Leu Asp Asp Thr Trp Glu Gly Lys Ile Tyr Arg Val Leu Ala Gly Asn
 245 250 255
 Pro Ala Lys His Asp Leu Asp Ile Lys Pro Thr Val Ile Ser His Arg
 260 265 270
 30 Leu His Phe Pro Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
 275 280 285
 Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
 290 295 300
 Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
 305 310 315 320
 40 Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
 325 330 335
 Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
 340 345 350
 45 Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
 355 360 365
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	Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu	
	370	380
5	Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala	
	385	400
	Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu	
	405	415
10	Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val	
	420	430
	Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu	
15	435	445
	Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr	
	450	460
20	His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val	
	465	480
	Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile	
	485	495
25	Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro	
	500	510
	Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val	
30	515	525
	Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala	
	530	540
35	Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu	
	545	560
	Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg	
	565	575
40	Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile	
	580	590
	Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp	
45	595	605
	Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp	
	610	620

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Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Pro Leu Ala Pro
625 630 635 640

5 Gly Leu Thr Lys Gly Ile Leu Gly Phe Val Phe Thr Leu Thr Val Pro
645 650 655

Gly Lys Pro Pro Arg Glu Asp Leu Lys Xaa Glu Phe
660 665

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(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

25

GCACCCGGGA TCCCGTCAGG CCCCTC

27

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

- (ii) MOLECULE TYPE: DNA (genomic)

35

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

40

GCACCCGGGC TCCCTCTGA GCTTCCT

27

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2238 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

5	ATGCACCTGA TACCCCATTTG GATCCCCCTG GTCGCCAGCC TCGGCCTGCT CGCCGGCGGC	60
10	TCGTCCCGCT CCGCCGCCGA GGAAGCTTTC GACCTCTGGA ACGAATGCGC CAAAGCCTGC	120
	GTGCTCGACC TCAAGGACGG CGTGCGTTCC AGCCGCATGA GCGTCGACCC GGCCATCGCC	180
	GACACCAACG GCCAGGGCGT GCTGCACTAC TCCATGGTCC TGGAGGGCGG CAACGACGCG	240
15	CTCAAGCTGG CCATCGACAA CGCCCTCAGC ATCACCAGCG ACGGCCTGAC CATCCGCCTC	300
	GAAGGCGGCG TCGAGCCGAA CAAGCCGGTG CGCTACAGCT ACACGCGCCA GGC GCGCGGC	360
20	AGTTGGTCGC TGAAGTGGCT GGTACCGATC GGCCACGAGA AGCCCTCGAA CATCAAGGTG	420
	TTCATCCACG AACTGAACGC CGGCAACCAG CTCAGCCACA TGTCGCCGAT CTACACCATC	480
	GAGATGGGCG ACGAGTTGCT GGCGAAGCTG GCGCGCGATG CCACCTTCTT CGTCAGGGCG	540
25	CACGAGAGCA ACGAGATGCA GCCGACGCTC GCCATCAGCC ATGCCGGGGT CAGCGTGGTC	600
	ATGGCCCCAGA CCCAGCCGCG CCGGGAAAAG CGCTGGAGCG AATGGGCCAG CGGCAAGGTG	660
30	TTGTGCCTGC TCGACCCGCT GGACGGGGTC TACAACTACC TCGCCCAGCA ACGCTGCAAC	720
	CTCGACGATA CCTGGGAAGG CAAGATCTAC CGGGTGCTCG CCGGCAACCC GGCGAAGCAT	780
	GACCTGGACA TCAAACCCAC GGTATCAGT CATCGCCTGC ACTTTCCCGA GGGCGGCAGC	840
35	CTGGCCGCGC TGACCGCGCA CCAGGCTTGC CACCTGCCGC TGGAGACTTT CACCCGTCAT	900
	CGCCAGCCGC GCGGCTGGGA ACAACTGGAG CAGTGC GGCT ATCCGGTGCA GCGGCTGGTC	960
40	GCCCTCTACC TGGCGGCGCG GCTGTCGTGG AACCAGGTCG ACCAGGTGAT CCGCAACGCC	1020
	CTGGCCAGCC CCGGCAGCGG CGGCGACCTG GGCGAAGCGA TCCGCGAGCA GCCGGAGCAG	1080
	GCCCCTCTGG CCCTGACCCT GGCCGCCGCC GAGAGCGAGC GCTTCGTCCG GCAGGGCACC	1140
45	GGCAACGACG AGGCCGGCGC GGCCAACGCC GACGTGGTGA GCCTGACCTG CCCGGTCGCC	1200
	GCCGGTGAAT GCGCGGGCCC GGCGGACAGC GGCGACGCC TGCTGGAGCG CAACTATCCC	1260

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ACTGGCGCGG AGTTCCTCGG CGACGGCGGC GACGTCAGCT TCAGCACCCG CGGCACGCAG 1320
AAGTGGACGG TGGAGCGGCT GCTCCAGGCG CACCGCCAAC TGGAGGAGCG CGGCTATGTG 1380
TTCGTCGGCT ACCACGGCAC CTTCTCGAA GCGGCGCAA GCATCGTCTT CGGCGGGGTG 1440
CGCGCGCGCA GCCAGGACCT CGACGCGATC TGGCGCGGTT TCTATATCGC CGGCGATCCG 1500
GCGCTGGCCT ACGGCTACGC CCAGGACCAG GAACCCGACG CACGCGGCCG GATCCGCAAC 1560
GGTGCCCTGC TGCGGGTCTA TGTGCCGCGC TCGAGCCTGC CGGGCTTCTA CCGCACCAGC 1620
CTGACCCTGG CCGCGCCGGA GCGGCGGGC GAGGTCGAAC GGCTGATCGG CCATCCGCTG 1680
CCGCTGCGCC TGGACGCCAT CACCGGCCCC GAGGAGGAAG GCGGGCGCCT GGAGACCATT 1740
CTCGGCTGGC CGCTGGCCGA GCGCACCGTG GTGATTCCCT CGGCGATCCC CACCGACCCG 1800
CGCAACGTCG GCGGCGACCT CGACCCGTCC AGCATCCCCG ACAAGGAACA GCGGATCAGC 1860
GCCCTGCCGG ACTACGCCAG CCAGCCCGGC AAACCGCCGC GCGAGGACCC GCTAGCACCC 1920
GGGATCCCGT CAGGCCCCCT CAAAGCCGAG ATCGCACAGA GACTTGAAGA TGTCTTTGCA 1980
GGGAAGAACA CCGATCTTGA GGTTCATG GAATGGCTAA AGACAAGACC AATCCTGTCA 2040
CCTCTGACTA AGGGGATTTT AGGATTTGTG TTCACGCTCA CCGTGCCCAG TGAGCGAGGA 2100
CTGCAGCGTA GACGCTTTGT CCAAATGCC CTTAATGGGA ACGGGGATCC AAATAACATG 2160
GACAAAGCAG TTAAACTGTA TAGGAAGCTC AAGAGGGAGC CCGGGAAACC GCCGCGCGAG 2220
GACCTGAAGT AAGAATTC 2238

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 746 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

5	Met His Leu Ile Pro His Trp Ile Pro Leu Val Ala Ser Leu Gly Leu	1	5	10	15
	Leu Ala Gly Gly Ser Ser Ala Ser Ala Ala Glu Glu Ala Phe Asp Leu	20	25	30	
10	Trp Asn Glu Cys Ala Lys Ala Cys Val Leu Asp Leu Lys Asp Gly Val	35	40	45	
	Arg Ser Ser Arg Met Ser Val Asp Pro Ala Ile Ala Asp Thr Asn Gly	50	55	60	
15	Gln Gly Val Leu His Tyr Ser Met Val Leu Glu Gly Gly Asn Asp Ala	65	70	75	80
	Leu Lys Leu Ala Ile Asp Asn Ala Leu Ser Ile Thr Ser Asp Gly Leu	85	90	95	
20	Thr Ile Arg Leu Glu Gly Gly Val Glu Pro Asn Lys Pro Val Arg Tyr	100	105	110	
	Ser Tyr Thr Arg Gln Ala Arg Gly Ser Trp Ser Leu Asn Trp Leu Val	115	120	125	
25	Pro Ile Gly His Glu Lys Pro Ser Asn Ile Lys Val Phe Ile His Glu	130	135	140	
30	Leu Asn Ala Gly Asn Gln Leu Ser His Met Ser Pro Ile Tyr Thr Ile	145	150	155	160
	Glu Met Gly Asp Glu Leu Leu Ala Lys Leu Ala Arg Asp Ala Thr Phe	165	170	175	
35	Phe Val Arg Ala His Glu Ser Asn Glu Met Gln Pro Thr Leu Ala Ile	180	185	190	
	Ser His Ala Gly Val Ser Val Val Met Ala Gln Thr Gln Pro Arg Arg	195	200	205	
40	Glu Lys Arg Trp Ser Glu Trp Ala Ser Gly Lys Val Leu Cys Leu Leu	210	215	220	
45	Asp Pro Leu Asp Gly Val Tyr Asn Tyr Leu Ala Gln Gln Arg Cys Asn	225	230	235	240
	Leu Asp Asp Thr Trp Glu Gly Lys Ile Tyr Arg Val Leu Ala Gly Asn	245	250	255	
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55					

Pro Ala Lys His Asp Leu Asp Ile Lys Pro Thr Val Ile Ser His Arg
 260 265 270
 5 Leu His Phe Pro Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln
 275 280 285
 Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
 290 295 300
 10 Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
 305 310 315 320
 Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
 325 330 335
 15 Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
 340 345 350
 Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
 355 360 365
 20 Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
 370 375 380
 Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
 385 390 395 400
 25 Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
 405 410 415
 30 Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
 420 425 430
 Ser Phe Ser Thr Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
 435 440 445
 Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
 450 455 460
 40 His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
 465 470 475 480
 Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
 485 490 495
 45 Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
 500 505 510
 50
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	Asp	Ala	Arg	Gly	Arg	Ile	Arg	Asn	Gly	Ala	Leu	Leu	Arg	Val	Tyr	Val	
				515					520							525	
5	Pro	Arg	Ser	Ser	Leu	Pro	Gly	Phe	Tyr	Arg	Thr	Ser	Leu	Thr	Leu	Ala	
				530					535							540	
	Ala	Pro	Glu	Ala	Ala	Gly	Glu	Val	Glu	Arg	Leu	Ile	Gly	His	Pro	Leu	
				545				550					555			560	
10	Pro	Leu	Arg	Leu	Asp	Ala	Ile	Thr	Gly	Pro	Glu	Glu	Glu	Gly	Gly	Arg	
					565							570				575	
	Leu	Glu	Thr	Ile	Leu	Gly	Trp	Pro	Leu	Ala	Glu	Arg	Thr	Val	Val	Ile	
15					580							585				590	
	Pro	Ser	Ala	Ile	Pro	Thr	Asp	Pro	Arg	Asn	Val	Gly	Gly	Asp	Leu	Asp	
					595						600				605		
20	Pro	Ser	Ser	Ile	Pro	Asp	Lys	Glu	Gln	Ala	Ile	Ser	Ala	Leu	Pro	Asp	
					610						615				620		
	Tyr	Ala	Ser	Gln	Pro	Gly	Lys	Pro	Pro	Arg	Glu	Asp	Pro	Leu	Ala	Pro	
					625						630				635		
25	Gly	Ile	Pro	Ser	Gly	Pro	Leu	Lys	Ala	Glu	Ile	Ala	Gln	Arg	Leu	Glu	
					645							650				655	
	Asp	Val	Phe	Ala	Gly	Lys	Asn	Thr	Asp	Leu	Glu	Val	Leu	Met	Glu	Trp	
30					660							665				670	
	Leu	Lys	Thr	Arg	Pro	Ile	Leu	Ser	Pro	Leu	Thr	Lys	Gly	Ile	Leu	Gly	
					675							680				685	
35	Phe	Val	Phe	Thr	Leu	Thr	Val	Pro	Ser	Glu	Arg	Gly	Leu	Gln	Arg	Arg	
					690							695				700	
	Arg	Phe	Val	Gln	Asn	Ala	Leu	Asn	Gly	Asn	Gly	Asp	Pro	Asn	Asn	Met	
					705							710				715	
40	Asp	Lys	Ala	Val	Lys	Leu	Tyr	Arg	Lys	Leu	Lys	Arg	Glu	Pro	Gly	Lys	
					725							730				735	
	Pro	Pro	Arg	Glu	Asp	Leu	Lys	Xaa	Glu	Phe							
45					740							745					

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CTAGACTAGT CTAG

14

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGCGGCAGAA AGAGC

15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala Ala Asp
1 5 10 15

Ala Asp Thr Ile Cys
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGCAGAAAGA TGAAGGCAAA CCTACTGGTC CTGTTATGTG CACTTGCAGC TGCAGATGCA 60
GACACAATAT GC 72

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Arg Lys Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala
1 5 10 15

Ala Ala Asp Ala Asp Thr Ile Cys
20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 63 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGAAGGCAA ACCTACTGGT CCTGTTATGT GCACTTGCAG CTGCAGATGC AGACACAATA 60
TGA 63

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala Ala Asp
1 5 10 15
Ala Asp Thr Ile Xaa
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

His His Ala Asn Glu Asn Ile Phe Tyr Cys Pro Ile Ala Ile Met Ser
1 5 10 15
Ala Leu Ala Met Val Tyr Leu Gly Ala Lys Asp
20 25

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CACCATGCCA ATGAGAACAT CTTCTACTGC CCCATTGCCA TCATGTCAGC TCTAGCCATG 60
GTATACCTGG GTGCAAAAAG C 81

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

His His Ala Asn Glu Asn Ile Phe Tyr Cys Pro Ile Ala Ile Met Ser
1 5 10 15
Ala Leu Ala Met Val Tyr Leu Gly Ala Lys Ser
20 25

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 78 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGCAGAAAGA TGAAGGCAAA CCTACTGGTC CTGTTATGTG CACTTGCAGC TGCAGATGCA 60
 5 GACACAATAT GCATGATG 78

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Arg Lys Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala
 1 5 10 15

Ala Ala Asp Ala Asp Thr Ile Cys Met Met
 20 25

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGCATGAAGG CAAACCTACT GGTCTGTGA TGTGCACTTG CAGCTGCAGA TGCAGACACA 60
 45 ATATGCATGA TG 72

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala Ala Ala
1 5 10 15

Asp Ala Asp Thr Ile Cys Met Met
20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 90 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTATGCATGC ACCATGCCAA TGAGAACATC TTCTACTGCC CCATTGCCAT CATGTCAGCT 60
CTAGCCATGG TATACCTGGG TGCAAAAGAC 90

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Val	Cys	Met	His	His	Ala	Asn	Glu	Asn	Ile	Phe	Tyr	Cys	Pro	Ile	Ala
1				5					10					15	
Ile	Met	Ser	Ala	Leu	Ala	Met	Val	Tyr	Leu	Gly	Ala	Lys	Asp		
			20				25						30		

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 147 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATGAAGGCAA ACCTACTGGT CCTGTTATGT GCACTTGCAG CTGCAGATGC AGACACAATA	60
TGCCACCATG CCAATGAGAA CATCTTCTAC TGCCCCATTG CCATCATGTC AGCTCTAGCC	120
ATGGTATACC TGGGTGCAAA AGACAGC	147

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

5 Met Lys Ala Asn Leu Leu Val Leu Leu Cys Ala Leu Ala Ala Asp
1 5 10 15
Ala Asp Thr Ile Cys His His Ala Asn Glu Asn Ile Phe Tyr Cys Pro
20 25 30
10 Ile Ala Ile Met Ser Ala Leu Ala Met Val Tyr Leu Gly Ala Lys Asp
35 40 45
Ser

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

30 CCTATCAGAA ACGAATGGGG GTGCAGATGC AACGGTTCAA GCGCGAGGAC CTGAAGTAAG 60
AATTCGAGCT 70

35 (2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 2013 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

	ATGGCCGAGG AAGCTTTCGA CCTCTGGAAC GAATGCGCCA AAGCCTGCGT GCTCGACCTC	60
5	AAGGACGGCG TGC GTTCCAG CCGCATGAGC GTCGACCCGG CCATCGCCGA CACCAACGGC	120
	CAGGGCGTGC TGCACTACTC CATGGTCCTG GAGGGCGGCA ACGACGCGCT CAAGCTGGCC	180
10	ATCGACAACG CCCTCAGCAT CACCAGCGAC GGCCTGACCA TCCGCCTCGA AGGCGGCGTC	240
	GAGCCGAACA AGCCGGTGCG CTACAGCTAC ACGCGCCAGG CGCGCGGCGAG TTGGTCGCTG	300
	AACTGGCTGG TACCGATCGG CCACGAGAAG CCCTCGAACA TCAAGGTGTT CATCCACGAA	360
15	CTGAACGCCG GCAACCAGCT CAGCCACATG TCGCCGATCT ACACCATCGA GATGGGCGAC	420
	GAGTTGCTGG CGAAGCTGGC GCGCGATGCC ACCTTCTTCG TCAGGGCGCA CGAGAGCAAC	480
20	GAGATGCAGC CGACGCTCGC CATCAGCCAT GCCGGGTCA GCGTGGTCAT GGCCCAGACC	540
	CAGCCGCGCC GGGAAAAGCG CTGGAGCGAA TGGGCCAGCG GCAAGGTGTT GTGCCTGCTC	600
	GACCCGCTGG ACGGGGTCTA CAACTACCTC GCCCAGCAAC GCTGCAACCT CGACGATACC	660
25	TGGAAGGCA AGATCTACCG GGTGCTCGCC GGCAACCCGG CGAAGCATGA CCTGGACATC	720
	AAACCCACGG TCATCAGTCA TCGCCTGCAC TTTCCCGAGG GCGGCAGCCT GGCCGCGCTG	780
30	ACCGCGCACC AGGCTTGCCA CCTGCCGCTG GAGACTTTCA CCCGTCATCG CCAGCCGCGC	840
	GGCTGGGAAC AACTGGAGCA GTGCGGCTAT CCGGTGCAGC GGCTGGTCGC CCTCTACCTG	900
	GCGGCGCGGC TGTCGTGGAA CCAGGTCGAC CAGGTGATCC GCAACGCCCT GGCCAGCCCC	960
35	GGCAGCGGCG GCGACCTGGG CGAAGCGATC CGCGAGCAGC CGGAGCAGGC CCGTCTGGCC	1020
	CTGACCCTGG CCGCCGCCGA GAGCGAGCGC TTCGTCCGGC AGGGCACCGG CAACGACGAG	1080
40	GCCGGCGCGG CCAACGCCGA CGTGGTGAGC CTGACCTGCC CGGTCGCCGC CGGTGAATGC	1140
	GCGGGCCCGG CGGACAGCGG CGACGCCCTG CTGGAGCGCA ACTATCCCAC TGGCGCGGAG	1200
	TTCTCGGCG ACGGCGGCGA CGTCAGCTTC AGCACCCGCG GCAGTCTTCT AACCGAGGTC	1260
45	GAAACGTACG TTCTCTCTAT CATCCCGTCA GGCCCCCTCA AAGCCGAGAT CGCACAGAGA	1320
	CTTGAAGATG TCTTTCAGG GAAGAACACC GATCTTGAGG TTCTCATGGA ATGGCTAAAG	1380

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ACAAGACCAA TCCTGTCACC TCTGACTAAG GGGATTTTAG GATTTGTGTT CACGCTCACC 1440
 GTGCCCAGTG AGCGAGGACT GCAGCGTAGA CGCTTTGTCC AAAATGCCCT TAATGGGAAC 1500
 5 GGGGATCCAA ATAACATGGA CAAAGCAGTT AACTGTATA GGAAGCTCAA GAGGGAGATA 1560
 ACATTCCATG GGGCCAAAGA AATCTCACTC AGTTATTCTG CTGGTGCAC TGCCAGTTGT 1620
 10 ATGGGCCTCA TATACAACAG GATGGGGGCT GTGACCACTG AAGTGGCATT TGGCCTGGTA 1680
 TGTGCAACCT GTGAACAGAT TGCTGACTCC CAGCATCGGT CTCATAGGCA AATGGTGACA 1740
 ACAACCAACC CACTAATCAG ACATGAGAAC AGAATGGTTT TAGCCAGCAC TACAGCTAAG 1800
 15 GCTATGGAGC AAATGGCTGG ATCGAGTGAG CAAGCAGCAG AGGCCATGGA GGTGCTAGT 1860
 CAGGCTAGGC AAATGGTGCA AGCGATGAGA ACCATTGGGA CTCATCCTAG CTCCAGTGCT 1920
 20 GGTCTGAAAA ATGATCTTCT TGAAAAATTG CAGGCCTATC AGAAACGAAT GGGGGTGCAG 1980
 ATGCAACGGT TCAAGCGCGA GGACCTGAAG TAA 2013

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 671 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Met Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys
 1 5 10 15

Val Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp
 20 25 30

Pro Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met
 35 40 45

Val Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala
 50 55 60

Leu Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val
 65 70 75 80
 5 Glu Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly
 85 90 95
 Ser Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser
 100 105 110
 10 Asn Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser
 115 120 125
 His Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala
 130 135 140
 15 Lys Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn
 145 150 155 160
 20 Glu Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val
 165 170 175
 Met Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala
 180 185 190
 25 Ser Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn
 195 200 205
 Tyr Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys
 210 215 220
 30 Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile
 225 230 235 240
 35 Lys Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser
 245 250 255
 Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr
 260 265 270
 40 Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys
 275 280 285
 Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu
 290 295 300
 45 Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro
 305 310 315 320
 50
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	Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln	
	325	330 335
5	Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val	
	340	345 350
	Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val	
	355	360 365
10	Val Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala	
	370	375 380
	Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu	
15	385	390 395 400
	Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Ser Leu	
	405	410 415
20	Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro Ser Gly Pro	
	420	425 430
	Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe Ala Gly Lys	
	435	440 445
25	Asn Thr Asp Leu Glu Val Leu Met Glu Trp Leu Lys Thr Arg Pro Ile	
	450	455 460
	Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe Thr Leu Thr	
30	465	470 475 480
	Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val Gln Asn Ala	
	485	490 495
35	Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala Val Lys Leu	
	500	505 510
	Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala Lys Glu Ile	
	515	520 525
40	Ser Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met Gly Leu Ile	
	530	535 540
	Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe Gly Leu Val	
45	545	550 555 560
	Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg Ser His Arg	
	565	570 575

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5 Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu Asn Arg Met
580 585 590

Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met Ala Gly Ser
595 600 605

10 Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln Ala Arg Gln
610 615 620

Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser Ser Ser Ala
625 630 635 640

15 Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr Gln Lys Arg
645 650 655

Met Gly Val Gln Met Gln Arg Phe Lys Arg Glu Asp Leu Lys Xaa
660 665 670

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATACCCGCGG CATGGCGTCC CAAGGCACCA AACGGTCT

38

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

5 ATAGAATTCT TACTTCAGGT CCTCGCGATT GTCGTACTCC TCTGCATTGT CTCCGAAGAA 60
ATAAGATCCT TCATTACTCA T 81

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 2754 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATGGCCGAGG AAGCTTTCGA CCTCTGGAAC GAATGCGCCA AAGCCTGCGT GCTCGACCTC 60
AAGGACGGCG TCGTTCCAG CCGCATGAGC GTCGACCCGG CCATCGCCGA CACCAACGGC 120
25 CAGGGCGTGC TGCACTACTC CATGGTCCTG GAGGGCGGCA ACGACGCGCT CAAGCTGGCC 180
ATCGACAACG CCCTCAGCAT CACCAGCGAC GGCCTGACCA TCCGCCTCGA AGGCGGCGTC 240
GAGCCGAACA AGCCGGTGCG CTACAGCTAC ACGCGCCAGG CGCGCGGCAG TTGGTCGCTG 300
30 AACTGGCTGG TACCGATCGG CCACGAGAAG CCCTCGAACA TCAAGGTGTT CATCCACGAA 360
CTGAACGCCG GCAACCAGCT CAGCCACATG TCGCCGATCT ACACCATCGA GATGGGCGAC 420
35 GAGTTGCTGG CGAAGCTGGC GCGCGATGCC ACCTTCTTCG TCAGGGCGCA CGAGAGCAAC 480
GAGATGCAGC CGACGCTCGC CATCAGCCAT GCCGGGGTCA GCGTGGTCAT GGCCAGACC 540
CAGCCGCGCC GGGAAAAGCG CTGGAGCGAA TGGGCCAGCG GCAAGGTGTT GTGCCTGCTC 600
40 GACCCGCTGG ACGGGGTCTA CAACTACCTC GCCCAGCAAC GCTGCAACCT CGACGATACC 660
TGGAAGGCA AGATCTACCG GGTGCTCGCC GGCAACCCGG CGAAGCATGA CCTGGACATC 720
45 AAACCCACGG TCATCAGTCA TCGCCTGCAC TTTCCCGAGG GCGGCAGCCT GGCCGCGCTG 780
ACCGCGCACC AGGCTTGCCA CCTGCCGCTG GAGACTTTCA CCCGTCATCG CCAGCCGCGC 840

	GGCTGGGAAC AACTGGAGCA GTGCGGCTAT CCGGTGCAGC GGCTGGTCGC CCTCTACCTG	900
	GCGGCGCGGC TGTCGTGGAA CCAGGTCGAC CAGGTGATCC GCAACGCCCT GGCCAGCCCC	960
5	GGCAGCGGCG GCGACCTGGG CGAAGCGATC CGCGAGCAGC CGGAGCAGGC CCGTCTGGCC	1020
	CTGACCCTGG CCGCCGCCGA GAGCGAGCGC TTCGTCCGGC AGGGCACC GG CAACGACGAG	1080
10	GCCGCGCGGC CCAACGCCGA CGTGGTGAGC CTGACCTGCC CGGTCGCCGC CGGTGAATGC	1140
	GCGGGCCCGG CGGACAGCGG CGACGCCCTG CTGGAGCGCA ACTATCCCAC TGGCGCGGAG	1200
	TTCCTCGGCG ACGGCGGCGA CGTCAGCTTC AGCACCCGCG GCATGGCGTC CCAAGGCACC	1260
15	AAACGGTCTT ACGAACAGAT GGAGACTGAT GGAGAACGCC AGAATGCCAC TGAAATCAGA	1320
	GCATCCGTCG GAAAAATGAT TGGTGAATT GGACGATTCT ACATCCAAAT GTGCACAGAA	1380
20	CTTAACTCA GTGATTATGA GGGACGGTTG ATCCAAAACA GCTTAACAAT AGAGAGAATG	1440
	GTGCTCTCTG CTTTGTACGA AAGGAGAAAT AAATACCTGG AAGAATCC CAGTGCGGGG	1500
	AAGGATCCTA AGAAAACTGG AGGACCTATA TACAGAAGAG TAAACGGAAA GTGGATGAGA	1560
25	GAACTCATCC TTTATGACAA AGAAGAAATA AGGCGAATCT GGCGCCAAGC TAATAATGGT	1620
	GACGATGCAA CGGCTGGTCT GACTCACATG ATGATCTGGC ATTCCAATTT GAATGATGCA	1680
30	ACTTATCAGA GGACAAGGGC TCTTGTTCGC ACCGGAATGG ATCCCAGGAT GTGCTCTCTG	1740
	ATGCAAGGTT CAACTCTCCC TAGGAGGTCT GGAGCCGCAG GTGCTGCAGT CAAAGGAGTT	1800
	GGAACAATGG TGATGGAATT GGTCAGGATG ATCAAACGTG GGATCAATGA TCGGAACTTC	1860
35	TGGAGGGGTG AGAATGGACG AAAACAAGA ATTGCTTATG AAAGAATGTG CAACATTCTC	1920
	AAAGGGAAAT TTCAAAGTGC TGCACAAAAA GCAATGATGG ATCAAGTGAG AGAGAGCCGG	1980
40	GACCCAGGGA ATGCTGAGTT CGAAGATCTC ACTTTTCTAG CACGGTCTGC ACTCATATTG	2040
	AGAGGGTCGG TTGCTCACAA GTCCTGCCTG CCTGCCTGTG TGTATGGACC TGCCGTAGCC	2100
	AGTGGGTACG ACTTTGAAAG AGAGGGATAC TCTCTAGTCG GAATAGACCC TTTCAGACTG	2160
45	CTTCAAAACA GCCAAGTGTA CAGCCTAATC AGACCAAATG AGAATCCAGC ACACAAGAGT	2220
	CAACTGGTGT GGATGGCATG CCATTCTGCC GCATTTGAAG ATCTAAGAGT ATTGAGCTTC	2280

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ATCAAAGGGA CGAAGGTGGT CCCAAGAGGG AAGCTTTCCA CTAGAGGAGT TCAAATTGCT 2340
 TCCAATGAAA ATATGGAGAC TATGGAATCA AGTACACTTG AACTGAGAAG CAGGTACTGG 2400
 5 GCCATAAGGA CCAGAAGTGG AGGAAACACC AATCAACAGA GGGCATCTGC GGGCCAAATC 2460
 AGCATACAAC CTACGTTCTC AGTACAGAGA AATCTCCCTT TTGACAGAAC AACCGTTATG 2520
 10 GCAGCATTCA CTGGGAATAC AGAGGGGAGA ACATCTGACA TGAGGACCGA AATCATAAGG 2580
 ATGATGGAAA GTGCAAGACC AGAAGATGTG TCTTTCCAGG GGCAGGGGAGT CTTGAGCTC 2640
 TCGGACGAAA AGGCAGCGAG CCCGATCGTG CCTTCCTTTG ACATGAGTAA TGAAGGATCT 2700
 15 TATTTCTTCG GAGACAATGC AGAGGAGTAC GACAATCGCG AGGACCTGAA GTAA 2754

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 918 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Met Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys
 1 5 10 15
 Val Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp
 20 25 30
 Pro Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met
 35 40 45
 Val Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala
 50 55 60
 Leu Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val
 65 70 75 80
 Glu Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly
 85 90 95

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	Ser Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser	
	100	105 110
5	Asn Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser	
	115	120 125
	His Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala	
	130	135 140
10	Lys Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn	
	145	150 155 160
	Glu Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val	
	165	170 175
15	Met Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala	
	180	185 190
20	Ser Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn	
	195	200 205
	Tyr Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys	
	210	215 220
25	Ile Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile	
	225	230 235 240
	Lys Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser	
	245	250 255
30	Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr	
	260	265 270
35	Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys	
	275	280 285
	Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu	
	290	295 300
40	Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro	
	305	310 315 320
	Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln	
	325	330 335
45	Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val	
	340	345 350

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Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val
355 360 365

5 Val Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala
370 375 380

Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu
385 390 395 400

10 Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Met Ala
405 410 415

Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp Gly Glu
420 425 430

15 Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met Ile Gly
435 440 445

Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys Leu Ser
20 450 455 460

Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu Arg Met
465 470 475 480

25 Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu Glu His
485 490 495

Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile Tyr Arg
30 500 505 510

Arg Val Asn Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp Lys Glu
515 520 525

35 Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp Ala Thr
530 535 540

Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn Asp Ala
545 550 555 560

40 Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp Pro Arg
565 570 575

Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser Gly Ala
45 580 585 590

Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu Leu Val
595 600 605

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	Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg Gly Glu	
	610	620
5	Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn Ile Leu	
	625	630 635 640
	Lys Gly Lys Phe Gln Thr Ala Ala Gln Lys Ala Met Met Asp Gln Val	
	645	650 655
10	Arg Glu Ser Arg Asp Pro Gly Asn Ala Glu Phe Glu Asp Leu Thr Phe	
	660	665 670
	Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His Lys Ser	
15	675	680 685
	Cys Leu Pro Ala Cys Val Tyr Gly Pro Ala Val Ala Ser Gly Tyr Asp	
	690	695 700
20	Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe Arg Leu	
	705	710 715 720
	Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu Asn Pro	
	725	730 735
25	Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala Ala Phe	
	740	745 750
	Glu Asp Leu Arg Val Leu Ser Phe Ile Lys Gly Thr Lys Val Val Pro	
30	755	760 765
	Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn Glu Asn	
	770	775 780
35	Met Glu Thr Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg Tyr Trp	
	785	790 795 800
	Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg Ala Ser	
	805	810 815
40	Ala Gly Gln Ile Ser Ile Gln Pro Thr Phe Ser Val Gln Arg Asn Leu	
	820	825 830
	Pro Phe Asp Arg Thr Thr Val Met Ala Ala Phe Thr Gly Asn Thr Glu	
45	835	840 845
	Gly Arg Thr Ser Asp Met Arg Thr Glu Ile Ile Arg Met Met Glu Ser	
	850	855 860

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Ala Arg Pro Glu Asp Val Ser Phe Gln Gly Arg Gly Val Phe Glu Leu
865 870 875 880

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Ser Asp Glu Lys Ala Ala Ser Pro Ile Val Pro Ser Phe Asp Met Ser
885 890 895

Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr Asp Asn
900 905 910

10

Arg Glu Asp Leu Lys Xaa
915

(2) INFORMATION FOR SEQ ID NO:55:

15

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

20

- (ii) MOLECULE TYPE: DNA (genomic)

25

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ATACCCGCGG CATGGGTGCG AGAGCGTCGG TATAT

35

(2) INFORMATION FOR SEQ ID NO:56:

30

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

35

- (ii) MOLECULE TYPE: DNA (genomic)

40

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

ATAGAATTCT CATGTGACG AGGGGTCGCT GCCAAA

36

45

50

55

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2814 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

15	ATGAAAAAGA CAGCTATCGC GATTGCAGTG GCACTGGCTG GTTTCGCTAC CGTAGCGCAG	60
	GCCGCGAATT TGGCCGAAGA AGCTTTCGAC CTCTGGAACG AATGCGCCAA AGCCTGCGTG	120
20	CTCGACCTCA AGGACGGCGT GCGTTCCAGC CGCATGAGCG TCGACCCGGC CATCGCCGAC	180
	ACCAACGGCC AGGGCGTGCT GCACTACTCC ATGGTCCTGG AGGGCGGCAA CGACGCGCTC	240
	AAGCTGGCCA TCGACAACGC CCTCAGCATC ACCAGCGACG GCCTGACCAT CCGCCTCGAA	300
25	GGCGGCGTCG AGCCGAACAA GCCGGTGCGC TACAGCTACA CGCGCCAGGC GCGCGGCAGT	360
	TGGTCGCTGA ACTGGCTGGT ACCGATCGGC CACGAGAAGC CCTCGAACAT CAAGGTGTTC	420
30	ATCCACGAAC TGAACGCCGG CAACCAGCTC AGCCACATGT CGCCGATCTA CACCATCGAG	480
	ATGGGCGACG AGTTGCTGGC GAAGCTGGCG CGCGATGCCA CCTTCTTCGT CAGGGCGCAC	540
	GAGAGCAACG AGATGCAGCC GACGCTCGCC ATCAGCCATG CCGGGGTCAG CGTGGTCATG	600
35	GCCCAGACCC AGCCGCGCCG GGAAAAGCGC TGGAGCGAAT GGGCCAGCGG CAAGGTGTTG	660
	TGCCTGCTCG ACCCGCTGGA CGGGGTCTAC AACTACCTCG CCCAGCAACG CTGCAACCTC	720
40	GACGATACCT GGAAGGCAA GATCTACCGG GTGCTCGCCG GCAACCCGGC GAAGCATGAC	780
	CTGGACATCA AACCACGGT CATCAGTCAT CGCCTGCACT TTCCGAGGG CGGCAGCCTG	840
	GCCGCGTGA CCGCGCACCA GGCTTGCCAC CTGCCGCTGG AGACTTTCAC CCGTCATCGC	900
45	CAGCCGCGCG GCTGGGAACA ACTGGAGCAG TCGGGCTATC CGGTGCAGCG GCTGGTCGCC	960
	CTCTACCTGG CGGCGGGCT GTCGTGGAAC CAGGTCGACC AGGTGATCCG CAACGCCCTG	1020

	GCCAGCCCCG GCAGCGGCGG CGACCTGGGC GAAGCGATCC GCGAGCAGCC GGAGCAGGCC	1080
	CGTCTGGCCC TGACCCTGGC CGCCGCCGAG AGCGAGCGCT TCGTCCGGCA GGGCACCGGC	1140
5	AACGACGAGG CCGGCGCGGC CAACGCCGAC GTGGTGAGCC TGACCTGCCC GGTGCGCCGC	1200
	GGTGAATGCG CGGGCCCGGC GGACAGCGGC GACGCCCTGC TGGAGCGCAA CTATCCCACT	1260
10	GGCGCGGAGT TCCTCGGCGA CGGCGGCGAC GTCAGCTTCA GCACCCGCGG CATGGGTGCG	1320
	AGAGCGTCGG TATTAAGCGG GGGAGAATTA GATAAATGGG AAAAAATTCG GTTAAGGCCA	1380
	GGGGGAAAGA AACAATATAA ACTAAACAT ATAGTATGGG CAAGCAGGGA GCTAGAACGA	1440
15	TTCGCAGTTA ATCCTGGCCT TTTAGAGACA TCAGAAGGCT GTAGACAAAT ACTGGGACAG	1500
	CTACAACCAT CCCTTCAGAC AGGATCAGAA GAACTTAGAT CATTATATAA TACAATAGCA	1560
20	GTCCTCTATT GTGTGCATCA AAGGATAGAT GTAAAAGACA CCAAGGAAGC CTTAGATAAG	1620
	ATAGAGGAAG AGCAAAACAA AAGTAAGAAA AAGGCACAGC AAGCAGCAGC TGACACAGGA	1680
	AACAACAGCC AGGTCAGCCA AAATTACCCT ATAGTCAGA ACCTCCAGGG GCAAATGGTA	1740
25	CATCAGGCCA TATCACCTAG AACTTTAAAT GCATGGGTAA AAGTAGTAGA AGAGAAGGCT	1800
	TTCAGCCCAG AAGTAATACC CATGTTTTCA GCATTATCAG AAGGAGCCAC CCCACAAGAT	1860
30	TTAAATACCA TGCTAAACAC AGTGGGGGGA CATCAAGCAG CCATGCAAAT GTTAAAAGAG	1920
	ACCATCAATG AGGAAGCTGC AGAATGGGAT AGATTGCATC CAGTGCATGC AGGGCCTATT	1980
	GCACCAGGCC AGATGAGAGA ACCAAGGGGA AGTGACATAG CAGGAACTAC TAGTACCCTT	2040
35	CAGGAACAAA TAGGATGGAT GACACATAAT CCACCTATCC CAGTAGGAGA AATCTATAAA	2100
	AGATGGATAA TCCTGGGATT AAATAAAATA GTAAGAATGT ATAGCCCTAC CAGCATTCTG	2160
40	GACATAAGAC AAGGACCAAA GGAACCCCTT AGAGACTATG TAGACCGATT CTATAAACT	2220
	CTAAGAGCCG AGCAAGCTTC ACAAGAGGTA AAAAATTGGA TGACAGAAAC CTTGTTGGTC	2280
	CAAAATGCGA ACCCAGATTG TAAGACTATT TTAAAAGCAT TGGGACCAGG AGCGACACTA	2340
45	GAAGAAATGA TGACAGCATG TCAGGGAGTG GGGGGACCCG GCCATAAAGC AAGAGTTTTG	2400
	GCTGAAGCAA TGAGCCAAGT AACAAATCCA GCTACCATAA TGATACAGAA AGGCAATTTT	2460

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AGGAACCAAA GAAAGACTGT TAAGTGTTC AATTGTGGCA AAGAAGGGCA CATAGCCAAA 2520
 AATTGCAGGG CCCCTAGGAA AAAGGGCTGT TGGAAATGTG GAAAGGAAGG ACACCAAATG 2580
 AAAGATTGTA CTGAGAGACA GGCTAATTTT TTAGGGAAGA TCTGGCCTTC CCACAAGGGA 2640
 AGGCCAGGGA ATTTTCTTCA GAGCAGACCA GAGCCAACAG CCCACCAGA AGAGAGCTTC 2700
 AGGTTTGGGG AAGAGACAAC AACTCCCTCT CAGAAGCAGG AGCCGATAGA CAAGGAACTG 2760
 TATCCTTTAG CTTCCTCAG ATCACTCTTT GGCAGCGACC CCTCGTCACA ATGA 2814

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 938 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
 1 5 10 15
 Thr Val Ala Gln Ala Ala Asn Leu Ala Glu Glu Ala Phe Asp Leu Trp
 20 25 30
 Asn Glu Cys Ala Lys Ala Cys Val Leu Asp Leu Lys Asp Gly Val Arg
 35 40 45
 Ser Ser Arg Met Ser Val Asp Pro Ala Ile Ala Asp Thr Asn Gly Gln
 50 55 60
 Gly Val Leu His Tyr Ser Met Val Leu Glu Gly Gly Asn Asp Ala Leu
 65 70 75 80
 Lys Leu Ala Ile Asp Asn Ala Leu Ser Ile Thr Ser Asp Gly Leu Thr
 85 90 95
 Ile Arg Leu Glu Gly Gly Val Glu Pro Asn Lys Pro Val Arg Tyr Ser
 100 105 110
 Tyr Thr Arg Gln Ala Arg Gly Ser Trp Ser Leu Asn Trp Leu Val Pro
 115 120 125

5 Ile Gly His Glu Lys Pro Ser Asn Ile Lys Val Phe Ile His Glu Leu
 130 135 140

Asn Ala Gly Asn Gln Leu Ser His Met Ser Pro Ile Tyr Thr Ile Glu
 145 150 155 160

10 Met Gly Asp Glu Leu Leu Ala Lys Leu Ala Arg Asp Ala Thr Phe Phe
 165 170 175

Val Arg Ala His Glu Ser Asn Glu Met Gln Pro Thr Leu Ala Ile Ser
 180 185 190

15 His Ala Gly Val Ser Val Val Met Ala Gln Thr Gln Pro Arg Arg Glu
 195 200 205

Lys Arg Trp Ser Glu Trp Ala Ser Gly Lys Val Leu Cys Leu Leu Asp
 210 215 220

20 Pro Leu Asp Gly Val Tyr Asn Tyr Leu Ala Gln Gln Arg Cys Asn Leu
 225 230 235 240

25 Asp Asp Thr Trp Glu Gly Lys Ile Tyr Arg Val Leu Ala Gly Asn Pro
 245 250 255

Ala Lys His Asp Leu Asp Ile Lys Pro Thr Val Ile Ser His Arg Leu
 260 265 270

30 His Phe Pro Glu Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala
 275 280 285

Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg Gly
 290 295 300

35 Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala
 305 310 315 320

40 Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile
 325 330 335

Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala
 340 345 350

45 Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala
 355 360 365

Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu Ala
 370 375 380

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Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala Ala
 385 390 395 400
 5 Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu Arg
 405 410 415
 Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val Ser
 420 425 430
 10 Phe Ser Thr Arg Gly Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly
 435 440 445
 Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys
 450 455 460
 15 Gln Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg
 465 470 475 480
 Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln
 485 490 495
 20 Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu
 500 505 510
 Arg Ser Leu Tyr Asn Thr Ile Ala Val Leu Tyr Cys Val His Gln Arg
 515 520 525
 25 Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu
 530 535 540
 Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly
 545 550 555 560
 35 Asn Asn Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln
 565 570 575
 Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp
 580 585 590
 40 Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met
 595 600 605
 Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met
 610 615 620
 45 Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu
 625 630 635 640

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	Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His	
	645	650 655
5	Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp	
	660	665 670
	Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr	
	675	680 685
10	His Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile	
	690	695 700
	Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu	
15	705	710 715 720
	Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg	
	725	730 735
20	Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn	
	740	745 750
	Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys	
	755	760 765
25	Thr Ile Leu Lys Ala Leu Gly Pro Gly Ala Thr Leu Glu Glu Met Met	
	770	775 780
	Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu	
30	785	790 795 800
	Ala Glu Ala Met Ser Gln Val Thr Asn Pro Ala Thr Ile Met Ile Gln	
	805	810 815
35	Lys Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys	
	820	825 830
	Gly Lys Glu Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys	
	835	840 845
40	Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr	
	850	855 860
	Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly	
45	865	870 875 880
	Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro	
	885	890 895

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Glu Glu Ser Phe Arg Phe Gly Glu Glu Thr Thr Thr Pro Ser Gln Lys
 900 905 910

5 Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser
 915 920 925

10 Leu Phe Gly Ser Asp Pro Ser Ser Gln Xaa
 930 935

Claims

- 15 1. A hybrid protein comprising:
 - (a) a modified bacterial toxin that has a translocating domain, and
 - (b) a polypeptide or protein that is exogenous to an antigen-presenting cell,
 said hybrid capable of eliciting an immune response by cytotoxic T lymphocytes.
- 20 2. A hybrid protein comprising:
 - (a) a modified Pseudomonas exotoxin; and
 - (b) a polypeptide or protein that is exogenous to an antigen-presenting cell;
 said hybrid capable of eliciting an immune response by cytotoxic T lymphocytes.
- 25 3. A hybrid protein comprising:
 - (a) a modified Pseudomonas exotoxin; and
 - (b) a polypeptide or protein that is exogenous to an antigen-presenting cell;
 said hybrid capable of being at least partially presented on an antigen-presenting cell surface.
- 30 4. A hybrid protein comprising:
 - (a) a modified Pseudomonas exotoxin; and
 - (b) a polypeptide or protein of viral, parasitic or tumor origin;
 said hybrid capable of being at least partially presented on an antigen-presenting cell surface.
- 35 5. A hybrid protein comprising:
 - (a) a modified Pseudomonas exotoxin; and
 - (b) a polypeptide or protein of viral origin;
 said hybrid capable of being internalized by an antigen-presenting cell and further capable of being at least partially presented on the surface of said antigen-presenting cell.
- 40 6. A hybrid protein comprising:
 - (a) a modified Pseudomonas exotoxin; and
 - (b) a polypeptide or protein of viral origin;
 said hybrid capable of being internalized by an antigen-presenting cell and further capable of being
- 45 processed for at least partial presentation on the surface of said antigen-presenting cell sufficiently to elicit an immune response by cytotoxic T lymphocytes.
7. The hybrid protein as claimed in claim 1, wherein said modified bacterial toxin further comprises a cellular recognition domain.
- 50 8. The hybrid protein as claimed in claim 2, wherein said modified Pseudomonas exotoxin lacks a functioning ADP ribosylating domain.
9. The hybrid protein as claimed in claim 2, wherein said modified Pseudomonas exotoxin comprises a cellular recognition domain and a translocating domain.
- 55 10. The hybrid protein as claimed in claim 2, wherein said modified Pseudomonas exotoxin comprises structural domains Ia, II and Ib.

11. The hybrid protein as claimed in claim 2, wherein said modified Pseudomonas exotoxin is arranged on the amino-terminal side of said hybrid and said polypeptide is arranged on the carboxyl-terminal side of said hybrid protein.
- 5 12. The hybrid protein as claimed in claim 2, wherein said polypeptide or protein is a viral protein fragment.
13. The hybrid protein as claimed in claim 12, wherein said viral protein fragment comprises the matrix protein of influenza A virus.
- 10 14. The hybrid protein as claimed in claim 12, wherein said viral protein fragment comprises residues 57 to 68 of the matrix protein of influenza A virus.
15. The hybrid protein as claimed in claim 12, wherein said viral protein fragment is sufficiently specific to bind to HLA-A2.
- 15 16. The hybrid protein as claimed in claim 12, wherein said viral protein fragment comprises the nucleoprotein of influenza A virus.
17. The hybrid protein as claimed in claim 12, wherein said viral protein fragment comprises the gag protein of human immunodeficiency virus-1.
- 20 18. The hybrid protein as claimed in claim 1, wherein said polypeptide or protein is an antigen for use as a vaccine.
- 25 19. The hybrid protein as claimed in claim 18, wherein said antigen for use as a vaccine is a viral antigen.
20. The hybrid protein as claimed in claim 19, wherein said viral antigen is a conserved viral protein.
21. The hybrid as claimed in claim 11 additionally comprising the peptide sequence Arg Glu Asp Leu Lys arranged on the carboxyl-terminal end of said polypeptide.
- 30 22. The hybrid protein as claimed in claim 21, and having the sequence described in Sequence ID No 35 or 38.
- 35 23. The hybrid protein as claimed in claim 8, wherein said Pseudomonas exotoxin further comprises an antigen peptide sequence inserted into structural domain III of said Pseudomonas exotoxin whose structural domain III cannot function as an ADP ribosylation domain.
- 40 24. The hybrid protein as claimed in claim 23, and having the sequence described in Sequence ID No. 19.
25. The hybrid protein as claimed in claim 23, and having the sequence described in Sequence ID No. 22.
- 45 26. A vaccine comprising a pharmaceutically acceptable carrier and an amount of the hybrid protein as claimed in claim 1 sufficient to elicit an immune response by cytotoxic T lymphocytes.
27. The vaccine as claimed in claim 26, wherein said hybrid protein comprises a modified Pseudomonas exotoxin and the matrix protein of influenza A virus.
- 50 28. The vaccine as claimed in claim 26, wherein said hybrid protein comprises a modified Pseudomonas exotoxin and residues 57 to 68 of the matrix protein of influenza A virus.
29. The vaccine as claimed in claim 26, wherein said hybrid protein comprises a modified Pseudomonas exotoxin and the nucleoprotein of influenza A.
- 55 30. The vaccine as claimed in claim 26, wherein said hybrid protein comprises a modified Pseudomonas exotoxin and the gag protein of human immunodeficiency virus-1.

31. The vaccine as claimed in claim 26, sufficient to immunize a host against influenza, acquired immunodeficiency syndrome, human papilloma virus, cytomegalovirus, Epstein-Barr virus, Rota virus, or respiratory syncytial virus.

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Pseudomonas Exotoxin

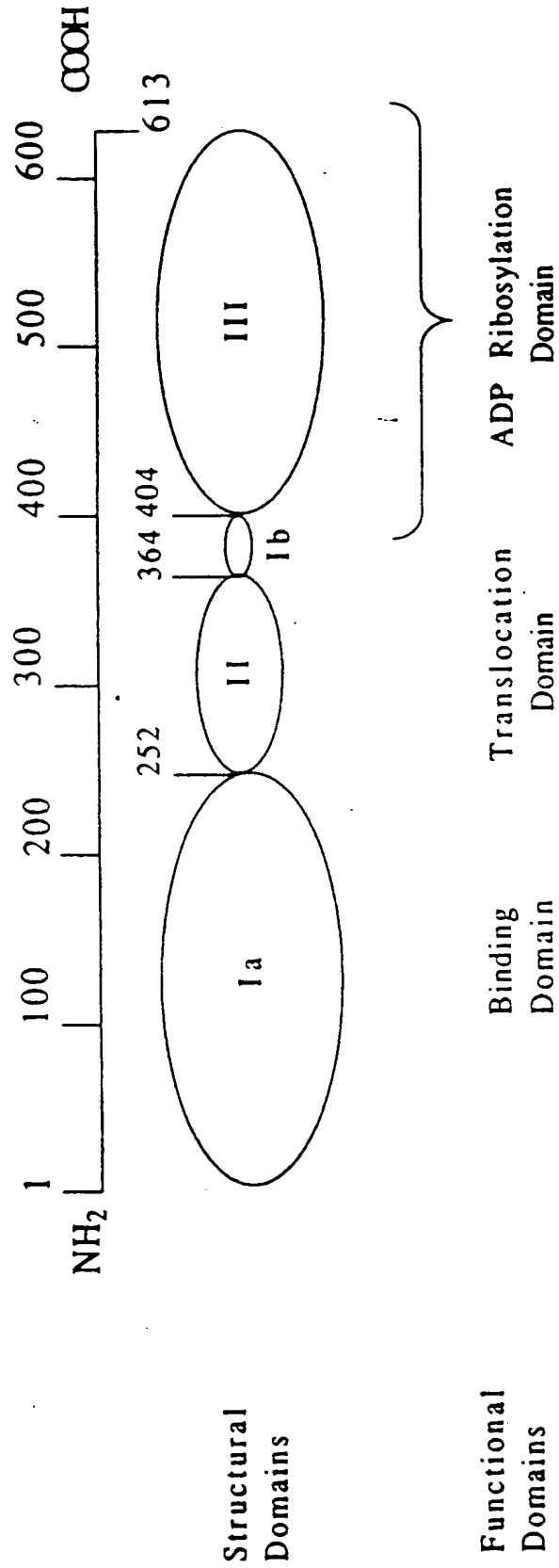


FIG. 1

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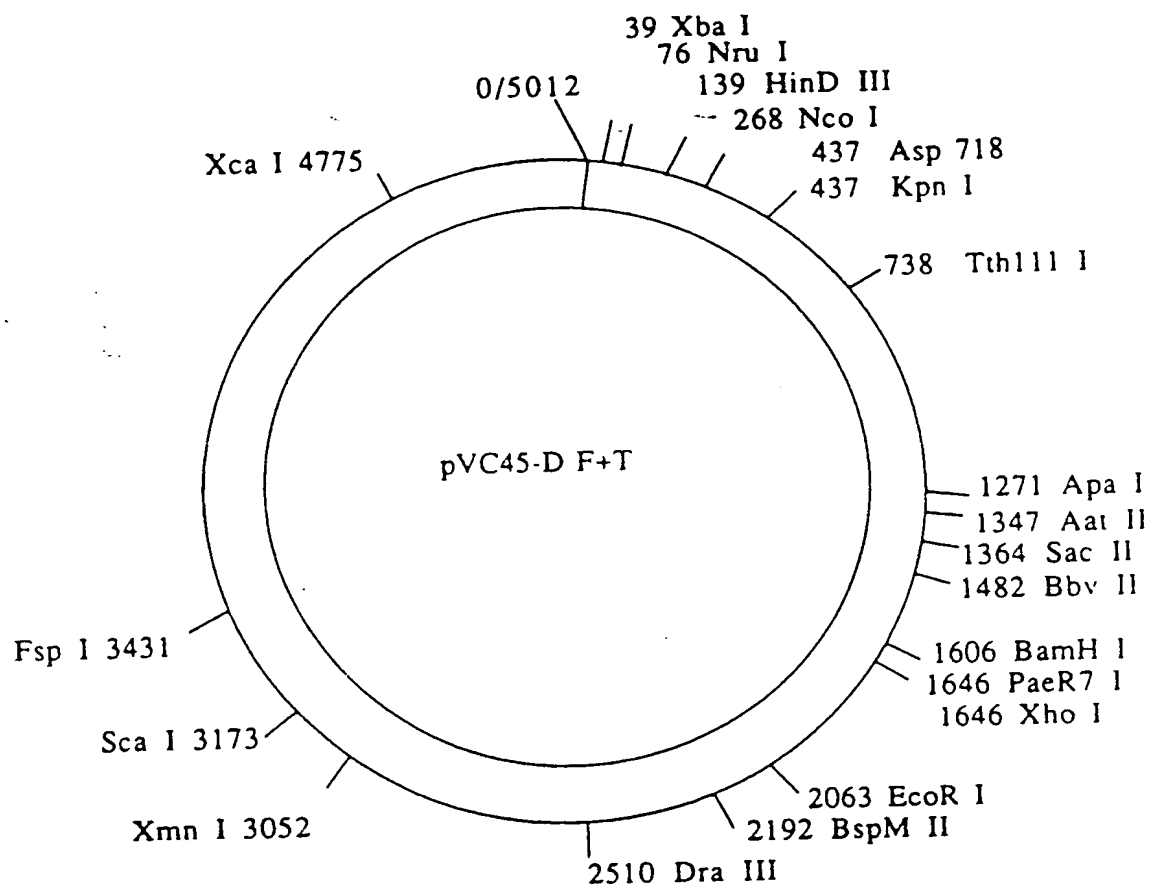


FIG. 2

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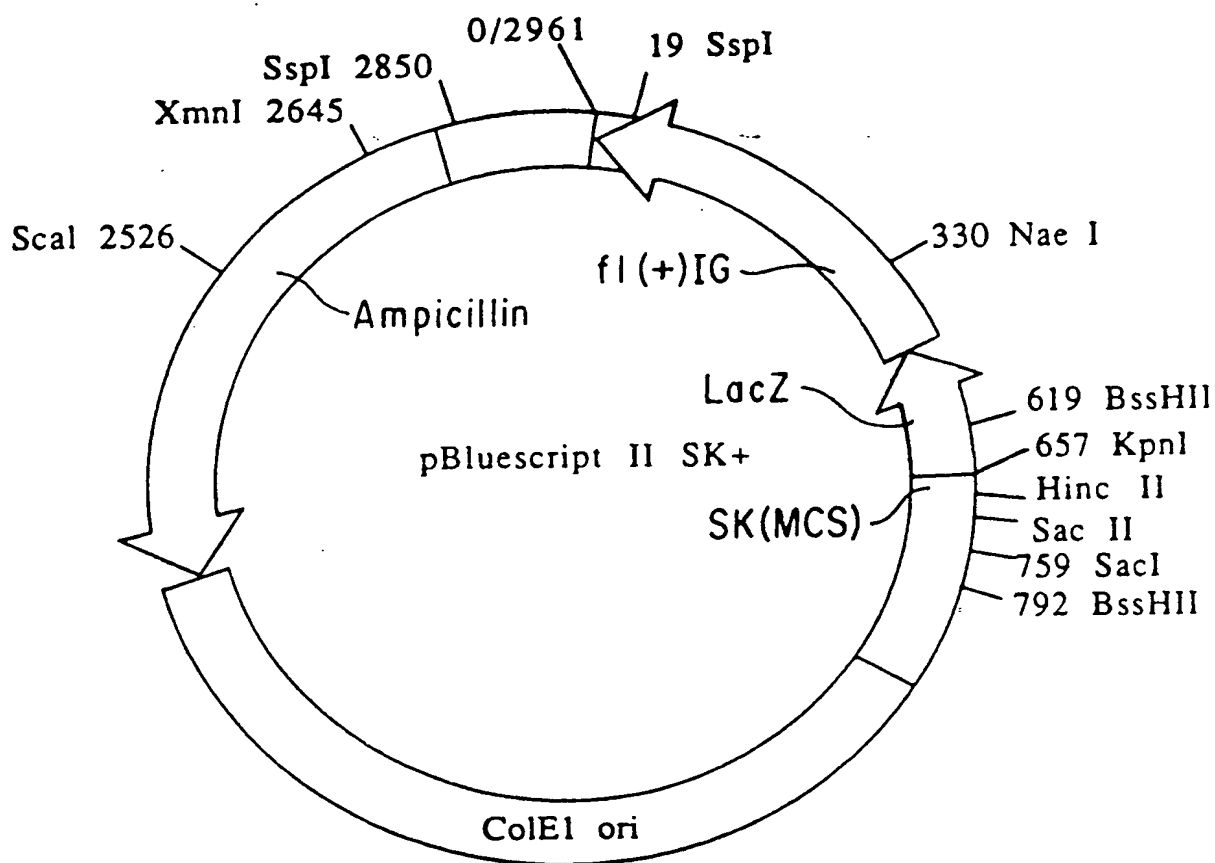


FIG. 3

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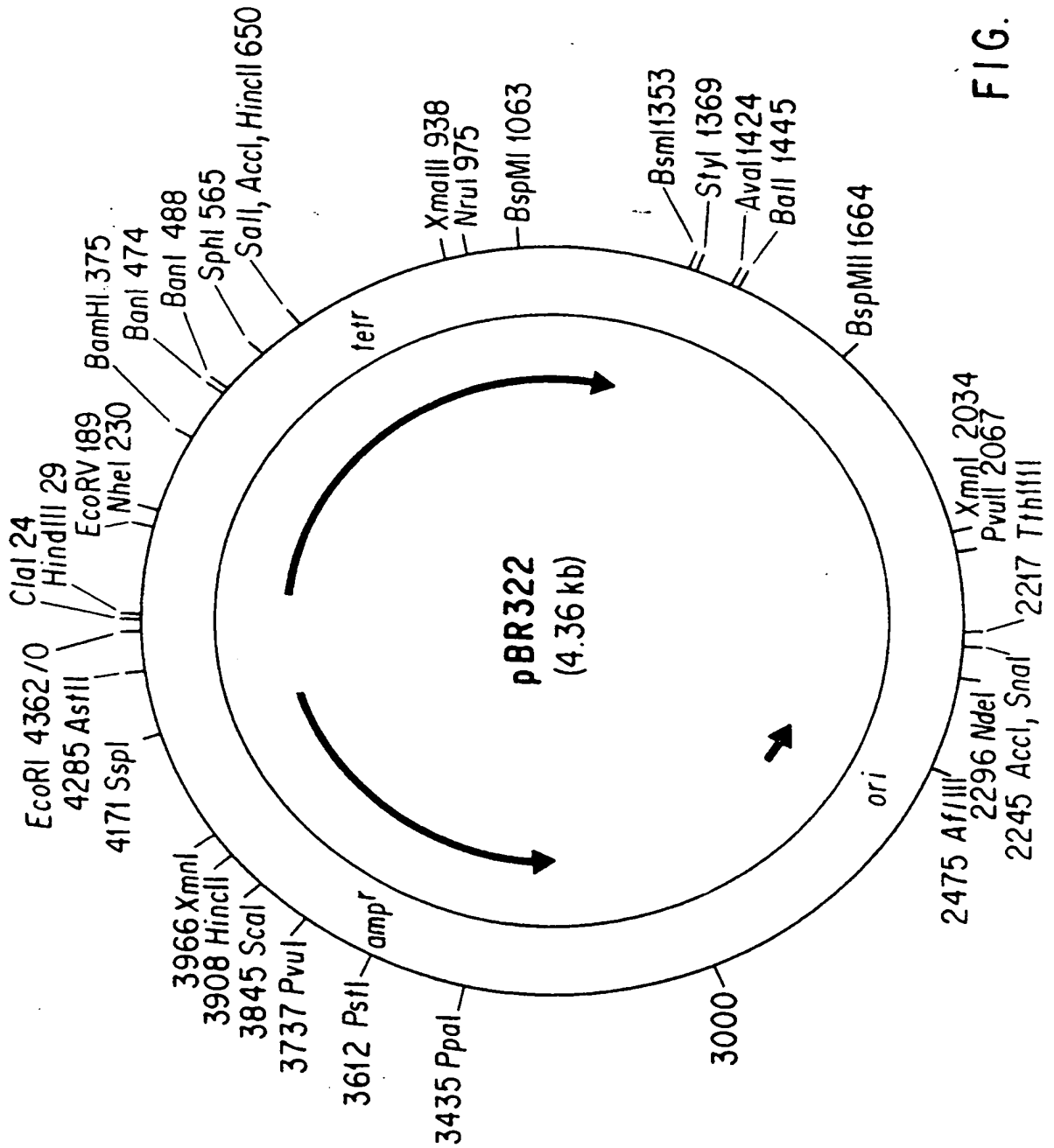


FIG. 4

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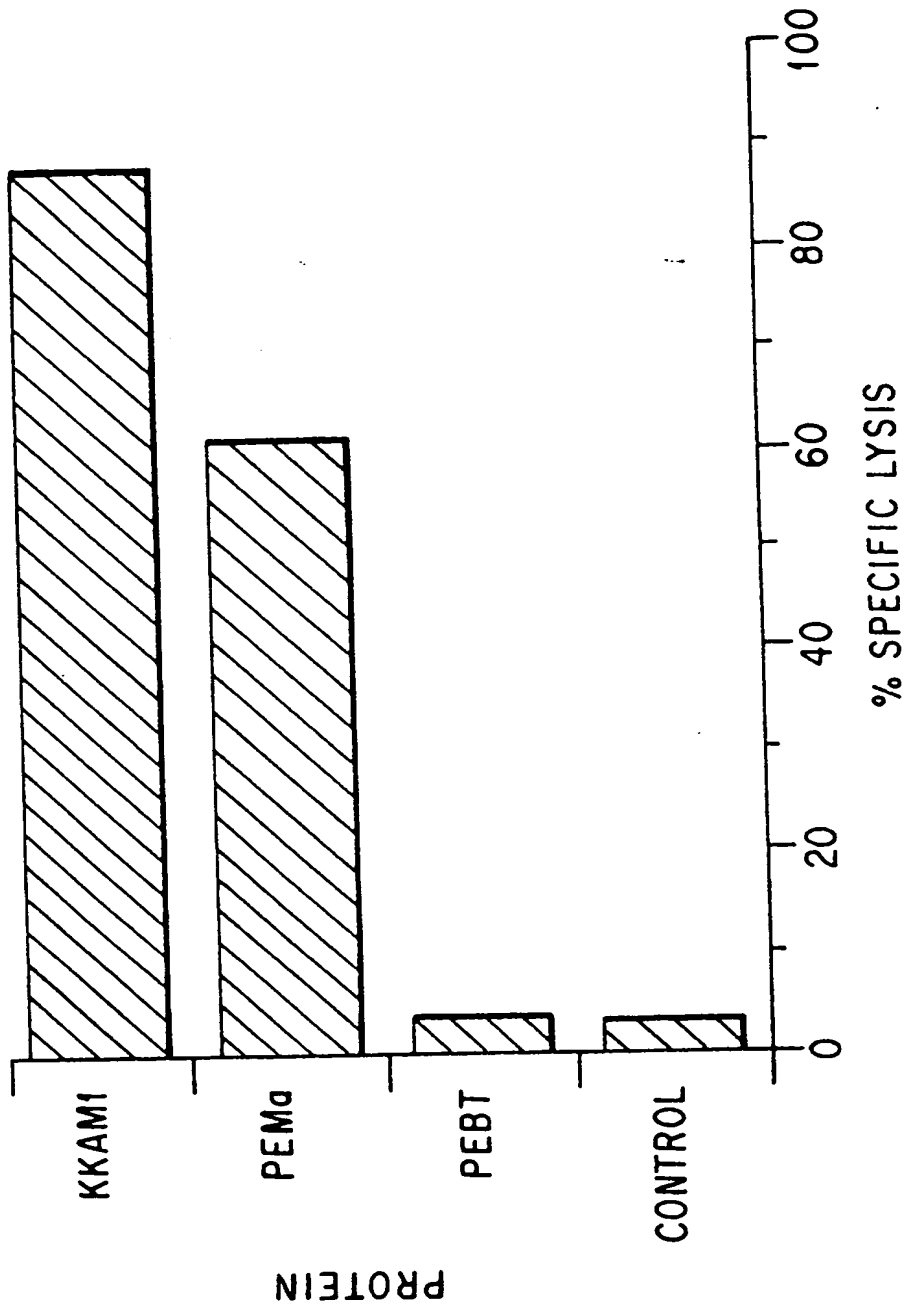


FIG. 5

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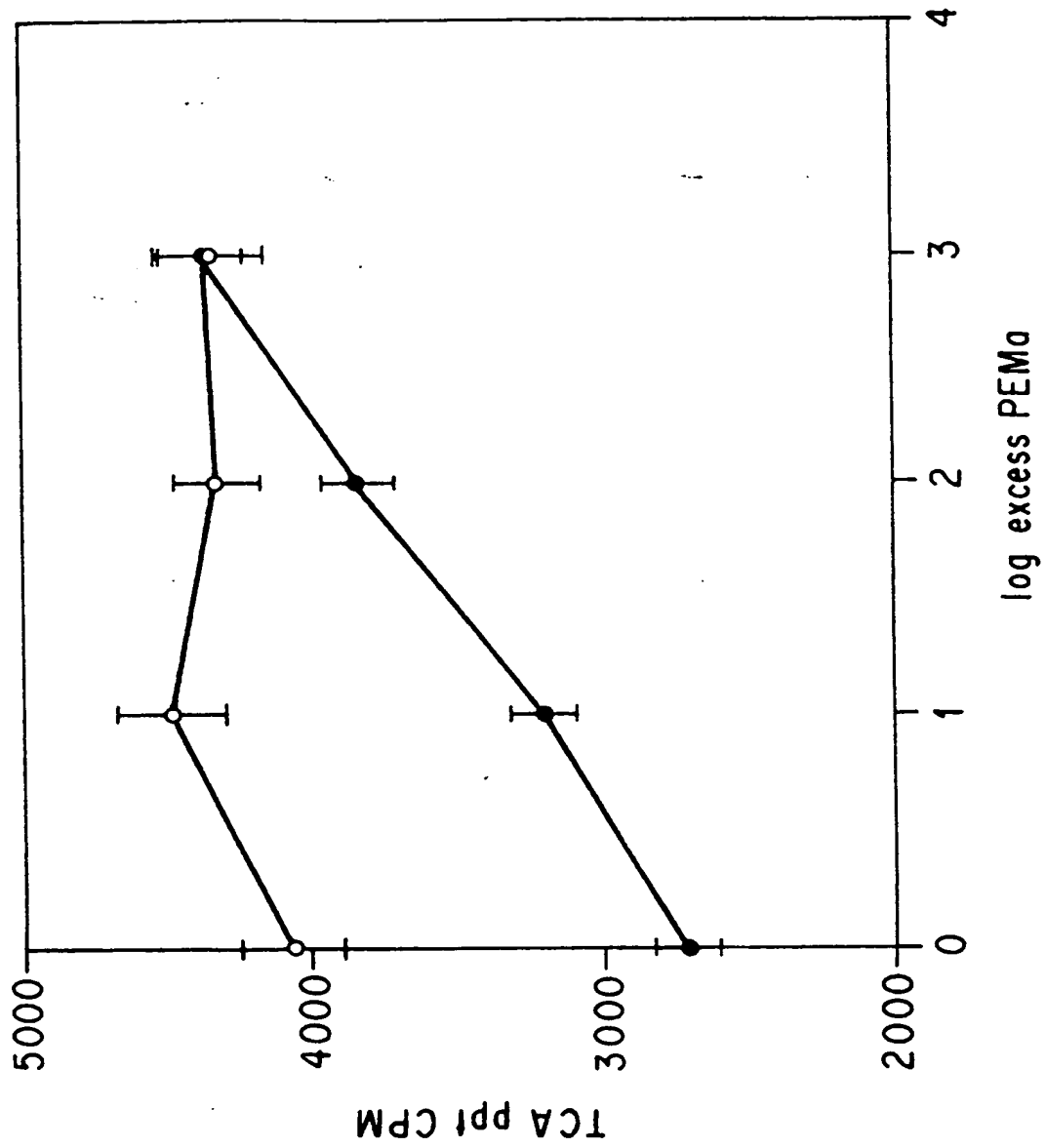


FIG. 6

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(C07K14/11)-(C07K14/16)-(C07K14/21-
[M07K201:02]-[M07K201:00]-
[M07K207:00]-[M07K211:00]-
[M07K211:02]-[M12N211:02]-
- 3- *- C07K14/21.



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//C12N15/62**

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(71) Applicant: **MERCK & CO. INC.**
126, East Lincoln Avenue
P.O. Box 2000
Rahway
New Jersey 07065-0900 (US)

(72) Inventor: **Donnelly, John J.**
1505 Brierwood Road
Havertown, PA 19083 (US)
Inventor: **Liu, Margaret A.**
4 Cushman Rd.
Rosemont, PA 19010 (US)
Inventor: **Friedman, Arthur**
121 Froghollow Road
Churchville, PA 18966 (US)

Inventor: **Montgomery, Donna L.**
9 Hickory Lane
Chalfont, PA 18914 (US)
Inventor: **Hawe, Linda A.**
2610 Skippack Pike
Norristown, PA 19403 (US)
Inventor: **Oliff, Allen I.**
1412 Florence Drive
Gwynedd Valley, PA 19437 (US)
Inventor: **Shi, Xiao-Ping**
536 Winthrop Rd.
Collegeville, PA 19426 (US)
Inventor: **Ulmer, Jeffrey**
128 Dolly Circle
Chalfont, PA 18914 (US)
Inventor: **Marshall, Mark S.**
1519 Spruce Ct.
Carmel, Indiana 46032 (US)

(74) Representative: **Thompson, John Dr. et al**
Merck & Co., Inc.
European Patent Department
Terlings Park
Eastwick Road
Harlow, Essex CM20 2QR (GB)

(54) Cellular immunity vaccines from bacterial toxin-antigen conjugates.

(57) Recombinant hybrid proteins having two primary components. The first component is a modified bacterial toxin that has translocating ability, while the second component is a polypeptide or protein that is exogenous to an antigen-presenting cell. The hybrid has the ability to be internalized by an antigen-presenting cell, where the hybrid is subsequently processed and an antigenic segment of the hybrid presented on the surface of the antigen-presenting cell, where the segment elicits an immune response by cytotoxic T lymphocytes.

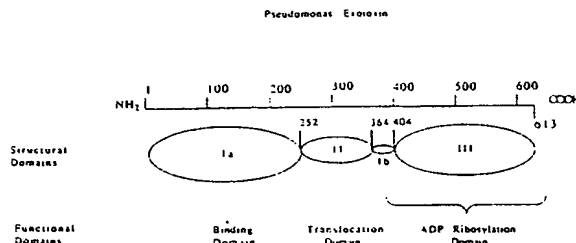


FIG. 1

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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 92 20 2660

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
A	WO-A-89 10971 (THE SECRETARY, U.S. DEPARTMENT OF COMMERCE) * claims * * figure 1 *	1-3,8,11	C07K13/00 A61K39/104 A61K39/145 A61K39/21 //C12N15/62
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European Patent
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EUROPEAN SEARCH REPORT

Application Number
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